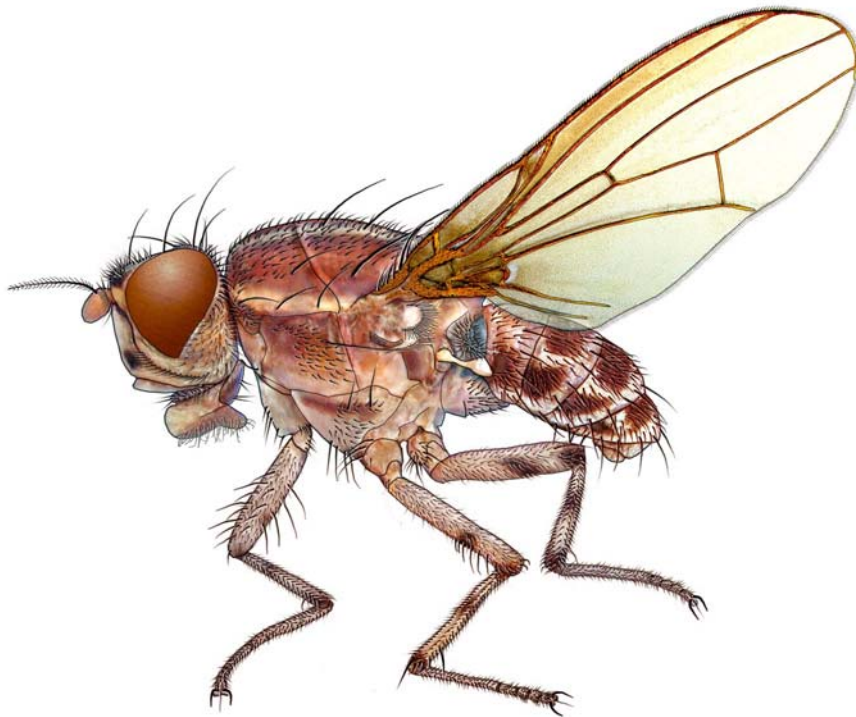


# **2003 PLANT PEST DIAGNOSTICS LABORATORY REPORT**



## PLANT PEST DIAGNOSTICS BRANCH ANNUAL REPORT 2003

**Umesh C. Kodira, Branch Chief**

### **Mission:**

The primary mission of the Plant Pest Diagnostics Center (PPDC) is to provide timely and accurate plant pest diagnostics in support of the pest prevention system in the California Department of Food and Agriculture. The branch also serves as a scientific resource and provides professional expertise to a number of clients including the Department, the United States Department of Agriculture, other federal and state agencies, County Agricultural Commissioners, the University of California Cooperative Extension, the agriculture industry and the public. Our scientists, technicians and support staff strive to provide excellence in service and leadership in plant pest diagnostics, and biosystematics.

This annual report is a summary of accomplishments in the past year. It provides updates on projects and highlights critical areas of research and new methodology in diagnostics and is by no means inclusive of all work performed at the PPDC.

### **Workload:**

The number of diagnostic samples processed in 2003 at PPDC includes:

Plant Taxonomy	3,284	Plant Pathology	88,233 <sup>a</sup>
Entomology	36,146		<sup>a</sup> (includes special projects)
Nematology	4,782	Seed sciences	3,067

*[Please note that the numbers cannot be compared between the different disciplines (labs) as an indication of workload.]*

### **Staffing Changes:**

Dr. Dennis E. Mayhew, the illustrious Branch Chief, decided to give up his scientific endeavors and move on to greener pastures. Dr. Mayhew accepted the position of Director of Plant Health and Pest Prevention Services effective May 2003. Dr. Umesh Kodira was appointed as the Branch Chief of Plant Pest Diagnostics Center effective November 2003. Dr. Mark Epstein and Dr. Shaun Winterton came on board with the PPDC as Associate Insect Biosystematists during the year. Dr. James Smith, Senior Plant Pathologist, retired from the Department after 37 years of dedicated service.

### **Research:**

The scientists at PPDC have been continuing to do research and publish scientific papers as part of the mission of this branch. In the past year, fifteen scientific papers were published and two posters were presented at professional meetings.

### **Other Projects:**

PPDC has been working with University of California, Davis during the past year in setting up the Western Plant Diagnostic Network, as part of an initiative for homeland security for agriculture. PPDC will provide the diagnostic support for the nine western

states. Staff from PPDC and the Department has also been instrumental in setting up the electronic database for the Network.

## **NEW FACES AND NEW PROJECTS AT THE PLANT PEST DIAGNOSTICS LABORATORY**

**Umesh Kodira, Branch Chief**

- Post Doctoral Researcher, Dr. Peter Kerr, has been working at the Plant Pest Diagnostics Center on a project involving the Systematics of the Tephritidae (identification of exotic fruit flies), using primarily molecular techniques coupled with classical identification methods. There are two major facets of the project. One is to possibly identify the exact origins of certain fruit fly pests, such as Mediterranean fruit fly (Medfly), either when they are found in California or when they are intercepted in quarantine. The second is the identification of larval fruit flies intercepted in quarantine using molecular techniques, particularly those of the *Bacterocera* (Oriental fruit fly) and *Anastrepha* (Mexican fruit fly) groups. Under current classical methods, this is nearly impossible for most species.
- Insect Biosystematist Dr. Marc Epstein has recently joined the PPDB staff, and brings with him many years experience in certain kinds of moths (the Limacodidae, the slug caterpillars) of agricultural significance. He is currently describing the morphology of a species in this group of moths that has recently been introduced in Hawaii and which we have already intercepted in quarantine coming from there. The moth is established in the eastern U.S., and is widespread throughout Asia and the Pacific Rim countries. It is a general feeder and is a serious enough pest to warrant having Mark publish an alert for use by our quarantine personnel and for the larval characters to be made available to other diagnosticians.
- The Nematology lab is currently involved in developing molecular markers for various hard to identify but common root-Knot nematodes. The projects involve raising various species on hydroponically grown tomato plants in the lab, then saving specimens for further molecular work by saving material in a cryogenic freezer. They are currently raising four common species out of some 65 known species.
- Dr. Fred Hrusa, Botanist, has been collaborating with scientists in Russia, the Ukraine, Uzbekistan, the Kew gardens in England and the Missouri Botanical gardens in a project trying to identify and trace the origins of the several species of Russian thistles (tumbleweeds) that occur in California. Russian thistle has been a serious weed pest all over the western U.S. for many years, and the Botanist and our Bio-Control unit are looking for answers on ways to control the pest with biological methods.
- Insect Biosystematist Ray Gill has been continuing a long term project on the identification of the various strains and related species of whiteflies called variously the sweet potato whitefly and the silver leaf whitefly. These groups of whiteflies are serious disease vectors of agricultural crops and have caused serious losses in California and all over the world. Collaboration with other identifiers and molecular workers includes scientists at the University of Arizona, Scotland, London, Taiwan, Spain, Israel and other localities.

# 2003 CDFA PLANT PEST DIAGNOSTICS BRANCH SAMPLE STATISTICS

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## ENTOMOLOGY LABORATORY Joanne Virone

Total Number of 2003 Entomology laboratory samples      36,146

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## PLANT TAXONOMY LABORATORY Fred Hrusa and Johanna Naughten

Total Number of 2003 Plant Taxonomy laboratory samples      3,284

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## NEMATOLOGY LABORATORY John Chitambar, Ke Dong, Robert Hackney and Rene Luna 4782

Total Number of 2003 Nematology laboratory samples      4,782  
Details of sample distribution to various subprograms is given in the Nematology Report Section.

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## SEED LABORATORY SAMPLE WORKLOAD 2003

Jim Effenberger, Elaine Harris, Don Joley, Deborah Meyer, Paul Peterson,  
Evelyn Ramos, Marian Stephenson, and Connie Weiner

Number of 2003 samples completed: 3067  
Number of tests completed on 2003 samples: 5521

Because individual samples received by the Seed Laboratory frequently require multiple tests, the number of tests conducted on the samples is a more realistic indicator of actual workload. Thus, the number of tests conducted is substantially greater than the number of actual samples completed. A detailed discussion of the numbers and types of samples as well as the numbers and types of tests conducted on those samples appears in the Seed Laboratory section of the 2003 annual report.

## PLANT PATHOLOGY LABORATORY

Jeanenne White

Samples in the Plant Pathology laboratory vary by projects and programs, some of which include partnered efforts with other CDFA branches. Sample numbers in Plant Pathology break down as follows:

### GENERAL PLANT PATHOLOGY

Total Diagnostic Samples	7,036
A-rated pathogens identified	53
Q-rated pathogens identified	286

### PLANT PATHOLOGY SPECIAL PROGRAMS

Seed Health Testing	470
Karnal Bunt Project	154
Sudden Oak Death (SOD)	1,866
Corn Stunt Project	1,973
Plum Pox Virus samples	23,750
Pierce's Disease samples	4,600
Nursery Virus Testing	
Stonefruit samples	47,684
Grapevine samples	2,694

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# Plant Taxonomy Laboratory

Staff:

Fred Hrusa

Johanna Naughton

Irene Wibawa

Yoshiko Kinmonth

Malin Kerr

PRELIMINARY ANALYSIS OF VARIATION IN THE NOXIOUS WEEDS  
SALSOLA L. SECT. KALI DUMORT, IN CALIFORNIA

G.F. HRUSA

BOTANY LABORATORY/HERBARIUM CDA

INTRODUCTION

The genus *Salsola* L., in the broad taxonomic sense is comprised of approximately 130 species, with a center of origin in the Mediterranean region, arid and coastal parts of Eurasia and in North, South and East Africa. Some weedy species are currently distributed worldwide. Various taxonomic treatments place these in several genera, but among the weedy *Salsola* in California three sections are represented; sect. *Caroxylon* (Thunb.) Fenzl, represented in California by *Salsola vermiculata*, sometimes segregated as the genus *Caroxylon* Thunb. Originally planted in a single site in 1969 Univ. of California rangeland trials in San Luis Obispo County, it escaped and spread during the next 15 years to cover more than 100 acres before a State and County effort was made to first contain and then eradicate it. Currently its numbers are low and eradication is expected to be successful. Section *Salsola*, with two species, has but a single annual species naturalized here, *Salsola soda*; and lastly sect. *Kali Dumort.* contains the remaining 3-5 annual taxa naturalized in California and with which we are specifically concerned in this report. All *Salsola* in California are agricultural and environmental pests, and only *S. soda* is not currently rated by CDFA. *Salsola vermiculata* is rated A, the remainder are rated C.

Worldwide, sect. *Kali* is comprised of approximately 15 annual species. Of these 6-8 have become naturalized in North America, with 3-5 in California. The most common in California (and North America) is a worldwide polymorphic entity, the taxonomy and nomenclature of which has been confused and in dispute for nearly a century in this country; indeed, this plant of Old World origin was twice described as distinct from North American weedy material, first by Thomas Walter (*Salsola caroliniana* Walt., *Flora Caroliniana*: 111 1788) and again by Ava Nelson (*Salsola pestifer* A. Nels., [New Man. Bot. Centr. Rocky Mt.](#) 169. 1909). That this situation has improved only slightly can be determined from the several names still being applied to this plant by both professional botanists and weed scientists. Fortunately, Mosyakin (1993) was able to clarify the affinity of the (lecto) type in the Linnaean Herbarium and thus establish nomenclatural priority as *Salsola tragus* L (Cent. Pl. 2: 13, 1756). Previously his plant has been known in California under several names; the synonyms *Salsola pestifer* (above), *S. ruthenica* Iljin (*Weed Fl. USSR* 2: 137. 1934), *S. australis* R. Br. (*Prodromus Florae Novae Hollandiae*, 27 Mar. 1810, 411), and *S. kali* var. *tenuifolia* Tausch. (*Flora* 11: 326, 1828). It has also been misapplied to *S. iberica* Senn. & Pau (*Bull. Acad. Geogr. Bot.* xviii. 476 (1908), a plant allied to *S. kali sensu stricto* and not *S. tragus*. *Salsola kali* L. *sensu stricto*, a maritime species naturalized in the southeastern U.S. (Mosyakin 1993) has also been accidentally misapplied (by leaving off the var. *tenuifolia*) to *S. tragus* in California. Any one of these names can still be found in reference to the tumbleweeds common throughout the Great Basin, the Southwest and California, and indeed some weed scientists in other states continue to use one or the other of these inappropriate names.

The taxonomic confusion centering on *Salsola tragus* extends also to the other *Salsola* species found in California. Specifically, the quite distinct *Salsola paulsenii* Litv., a species known primarily from east of the Sierra Nevada and Peninsular Range axes, has been applied to the same plants which have been called *S. tragus* et al.; T.C. Fuller felt some of these *S. tragus*-like specimens were hybrids between *S. tragus* (under other names) and *S. paulsenii* (notes in file at

CDA). However, no taxonomic or other scientific evidence was ever produced to indicate why these plants were thought to be hybrid derivatives of the two taxa above other than that they generally occur east of *Salsola tragus*, (most common in cismontane California, although it occurs in the Great Basin and eastward as well) and west of the generally transmontane *S. paulsenii*. In gestalt they look rather “intermediate”. This form was distinguished by Fuller from *S. paulsenii sensu stricto*, by the soft and obtuse-tipped “lax” tepals as in all other California *Salsola* sect. *Kali*. This is in contrast to those of typical *S. paulsenii* which are acicular and rigid. This form has been, and will be for this report, called *S. paulsenii* “lax”.

This state of taxonomic confusion existed in California primarily due to the lack of a modern revision of *Salsola* sect. *Kali*. *Salsola* is a difficult genus to classify, and sect. *Kali* particularly so, primarily due to the great variability in habit displayed among sympatric individuals, but also to the reduction of the plant body; there are few morphological characters available for comparison and with which to build a classification. Even the most current botanical manual for California, the Jepson Manual – Higher Plants of California (Hickman ed. Univ. of California Press, 1993), utilized the same characters and taxonomy (although not nomenclature) found in Munz’ A California Flora of 30 years previous. Even Mosyakin, in his revisions of North American *Salsola* (Ann. Mo. Bot. Garden, 83: 387-395, 1993 and in press) utilized the same morphological characteristics as had been used by virtually all previous workers, although modifying the nomenclature and taxonomy slightly, clarifying type applications and adjusting distributions. Finally, in 1999 S. Rilke published a monographic level revision of *Salsola* sect. *Kali* (in Bibliotheca Botanica, Stuttgart, 1999). This treatment moved beyond the standard taxonomic characteristics used in previous treatments of California *Salsola* which focused on winged fruit width and stem pubescence, to utilize fruiting calyx wing shapes and positions, plus anther, bract, and perianth size and form.

Currently, the Botany Laboratory, in collaboration with the Biocontrol program of the CDFA Integrated Pest Control Branch, Herbarium KW, Komorov Institute, Ukraine, USDA Biocontrol Program, Missouri Botanical Garden and Kew Gardens in Great Britain is working on a solution to the taxonomic confusion among *Salsola* taxa in California. Molecular, cytological and haplotype analyses have been carried out or are in progress by collaborators at Missouri, Kew and USDA; with typological application by Mosyakin at KW. The morphological analysis, which has been needed to determine application to types and develop identification keys, has been, and continues to be underway in the Botany Laboratory of the CDFA Plant Pest Diagnostics Branch. This report will focus on the problems so far found within the *Salsola tragus* “group” and provide some preliminary results. The problems are more than theoretical; biocontrol efforts require the ability to both recognize the taxa in the field and herbarium, and be able to communicate using botanical taxonomic nomenclature an accurate identity of the organisms from which potential control agents in their native ranges may be collected for testing against these California pests.

A molecular characterization of *Salsola tragus* s.l. in California (Ryan & Ayres, Can. J. Bot. 78: 59-67, 2000) revealed that two apparently “cryptic” and distantly related taxa were involved within plants originally identified as *S. tragus*. Some of the *Salsola tragus* were apparently the same as the widely naturalized *Salsola* in the U.S. Great Basin and in Europe, while the second was unidentified. An attempt in this laboratory in 1997 to apply a name to the unknown form, indicated clearly that the two types were not cryptic at all, but were morphologically distinct. However, no literature descriptions could be found to match the unknown taxon. After Rilke’s publication in 1999, further unsuccessful attempts were made to match the plants, both to the written descriptions and the excellent line drawings in her publication. By this time chromosome counts had been performed by collaborators, indicating the unknown type was diploid, while the typical *S. tragus* form was tetraploid. Further field work in California had begun to clarify the



distribution of the forms, yet the lack of a clear morphological treatment hampered both field recognition and comparison to general specimens and types held in herbaria in Russia and Ukraine. Thus a full morphological analysis of *Salsola* in California was needed before plants in Old World herbaria could be confidently matched to the unknown form, which by now was being referred to as “type B” versus the typical *Salsola tragus* which was given a working title of “type A.” These conventions will be followed throughout the remainder of this report.

## MATERIALS AND METHODS

Due to the extreme variability among populations and individuals of *Salsola* type A, a common-garden was established at the Plant Pest Diagnostics Center in Sacramento, in order to control for individual plastic response to environmental heterogeneity. Further, comparison among numerous populations and among the different taxa, not just types A and B, would be necessary to determine where the morphological limits could be drawn. Because most California *Salsola* are considered noxious weeds, a better defined taxonomy would be useful for all concerned. Therefore the study was expanded to initially include all known California *Salsola* sect. *Kali* taxa except *S. kali* ssp. *pontica*, material of which was unavailable for planting. Forms thought to possibly represent introgressant hybrid types (Type A Davis) or which were identified as distinct during molecular analyses but which did not match any common form (type C) were also included. Unfortunately, seeds available for germination and planting were not systematically sampled specifically for this project, rather study material was drawn from seed stocks available from CDFA Integrated Pest Control Biocontrol Program, with supplementary collections in Coalinga (type C), Barstow (*S. paulsenii* ‘typical’) and Mojave (*S. paulsenii* ‘lax’). For this study, however, it was felt that they covered enough of the geographic range to be useful for early comparisons. Each accession was given a working non-taxonomic name, provided in Table 1, where provenance data for accessions used in the common-garden study are also available. A total of 10 populations representing *S. tragus* type A, type B, type C, *Salsola paulsenii* ‘lax’ and *S. paulsenii* ‘typical’ were planted. Seed was sown directly into the ground in specified plots, clear of competing weeds, where the soil was relatively homogeneous. Samples were taken from the plants as mature calyx wings developed, pressed and dried. A minimum of four samples were taken from each replicate or if fewer were available all individuals were sampled. In order to determine their relative reactions to the end of the growing season, the sampled plants were allowed to senesce naturally. Fresh material from each common garden plot was sent to Dr. Fred Ryan at the USDA ARS SJVASC lab in Parlier, Ca. in order to confirm identity via isozyme profiling.

Quantitative data were taken under magnifications of 10X and 20X (depending upon the size of the structure) with an ocular micrometer. Each measurement was taken to a maximum of one-tenth of a millimeter. Four measurements per individual were taken for each character, and means calculated. Mean lengths and widths were then transformed into ratios to denote shape. Correlated widths or lengths (and thus redundant) were excluded from the analyses. Qualitative characters were placed into presence/absence categories and, for those statistical analyses that were to be combined with the quantitative data, were given a numerical status of 1 (one) or 0 (zero). All data were tested for outliers and to prevent larger structures from dominating the multivariate data, were converted using Gower’s ranging algorithm to a value between one (1) and zero (0). Preliminary statistical tests utilized various clustering algorithms and principal components analysis. Further multivariate & univariate analyses are currently in progress.

HRUSA TABLE 1: Study Material. Seeds were provided by CDFA, Integrated Pest Control, Biocontrol Program.

Taxon	Type	Pop. Code	County	Total #of individuals
<i>S. tragus</i>	A	Coal, Coal2, Coal3	Fresno	12
<i>S. tragus</i>	A	Davis, Davis2	Yolo	11
<i>S. tragus</i>	A	30 Acre, 30 Acre2, Fresno	Fresno	14
<i>S. tragus</i>	A	Bfield, Bfield2, Bfield3	Fresno	15
<i>Salsola</i> type B	B	SD, SD2, SD3	San Diego	18
<i>Salsola</i> type B	B	HCRL, HCRL2, HCRL3	Fresno	18
<i>Salsola</i> type B	B	SNella1, SNella2	Merced	13
<i>Salsola</i> type C	C	TypC 1, TypC2, TypC3	Fresno	11
<i>S. paulsenii</i> “lax”	SPL	LAX1, LAX2, LAX3	Kern	12
<i>S. paulsenii</i> “typical”	SPT	SPtyp1, SPtyp2, SPtyp3	San Bernardino	6

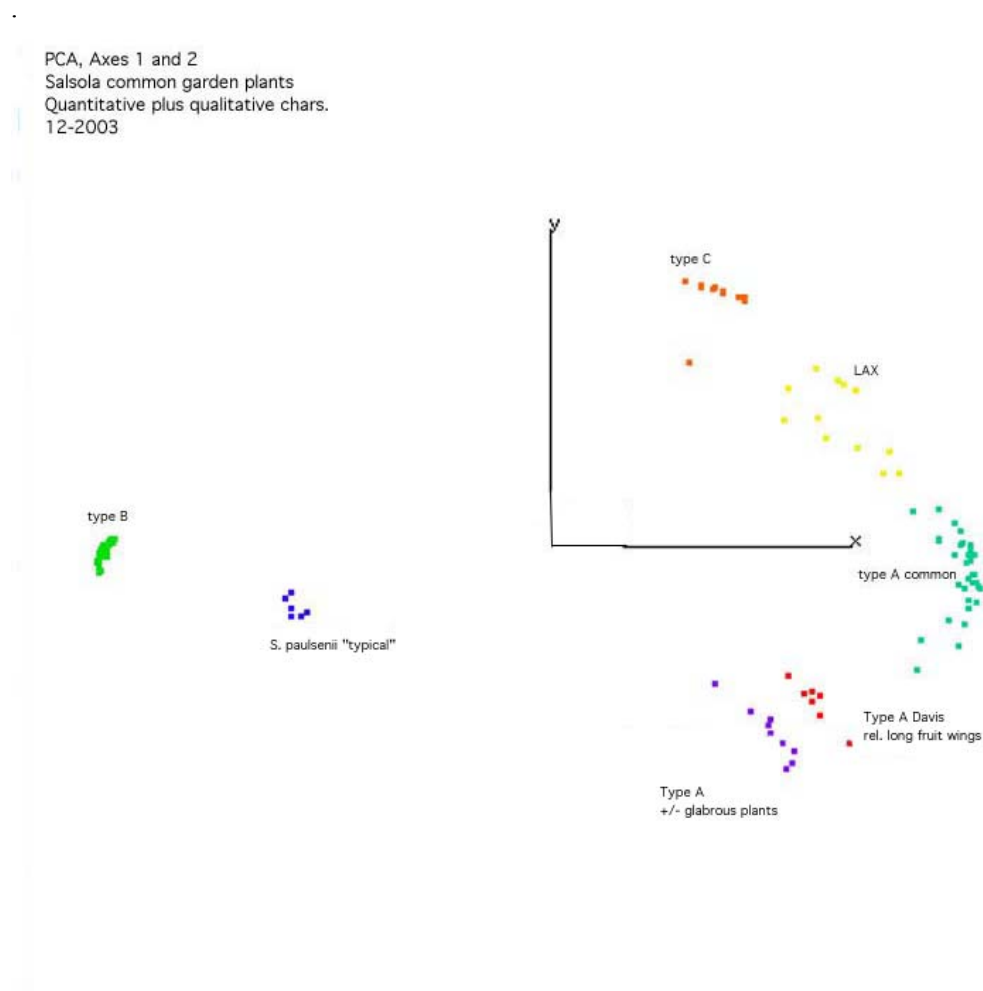
## RESULTS

Both UPGMA single-linkage cluster and principal components analysis (PCA) revealed a similar set of groupings, corresponding largely to the groups already recognized informally and described above. PCA ordinations are graphed in Fig 1, (components 1 and 2); and Fig. 2 (components 1 and 3); a cluster dendrogram is provided in Fig. 3. The exceptions were the groups ‘Type A, glabrous plants’ and ‘Type A Davis’. These groups had one or more characteristics that separated them from the remainder of the *S. tragus* type A plants; specifically they were plants that had either glabrous stems, a middle calyx wing slightly more rounded at the summit or both, as compared to the more common form of *S. tragus* type A which had hairy stems and a relatively flat or truncated wing summit (and thus a wider width to height ratio). These variants are interesting, but do not materially affect the analysis, and are probably an artifact of incomplete sampling of the highly variable type A.

The tight clustering shown by type B in the PCA and the short branches of the cluster dendrogram of the type B grouping reflect the lack of variation among type B individuals. This result is congruent with the molecular trees (see Ryan and Ayres, *op. cit.*) in which there was a similar lack of (molecular) variation among individuals (pers. comm. F. Ryan).

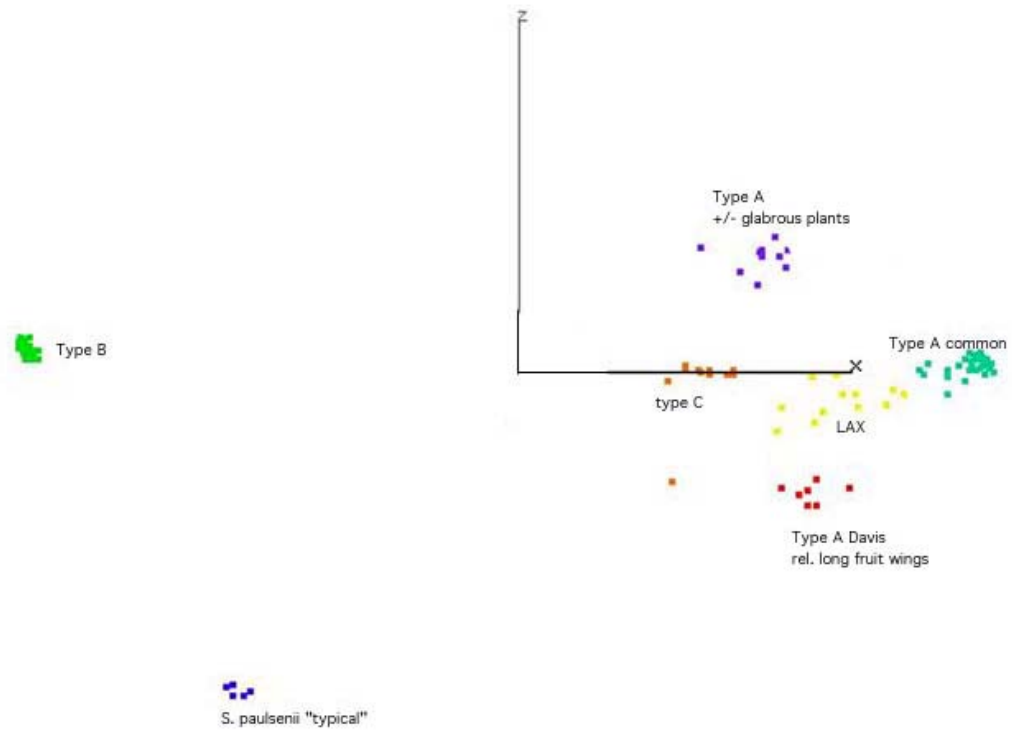
Although the sample size is inadequate to make firm judgments regarding the relative positioning of *S. paulsenii* ‘lax’ and *Salsola* type C, it is clear that ‘lax’ is not situated between its hypothesized parents, Type A and *S. paulsenii* ‘typical’. However, further populations of type C, found subsequent to the common-garden being established, suggest that this type is more variable than was accounted for in the single population available originally for study, and thus there will

be no further discussion of the forms within of type C and *S. paulsenii* 'lax' until more extensive comparisons are undertaken.

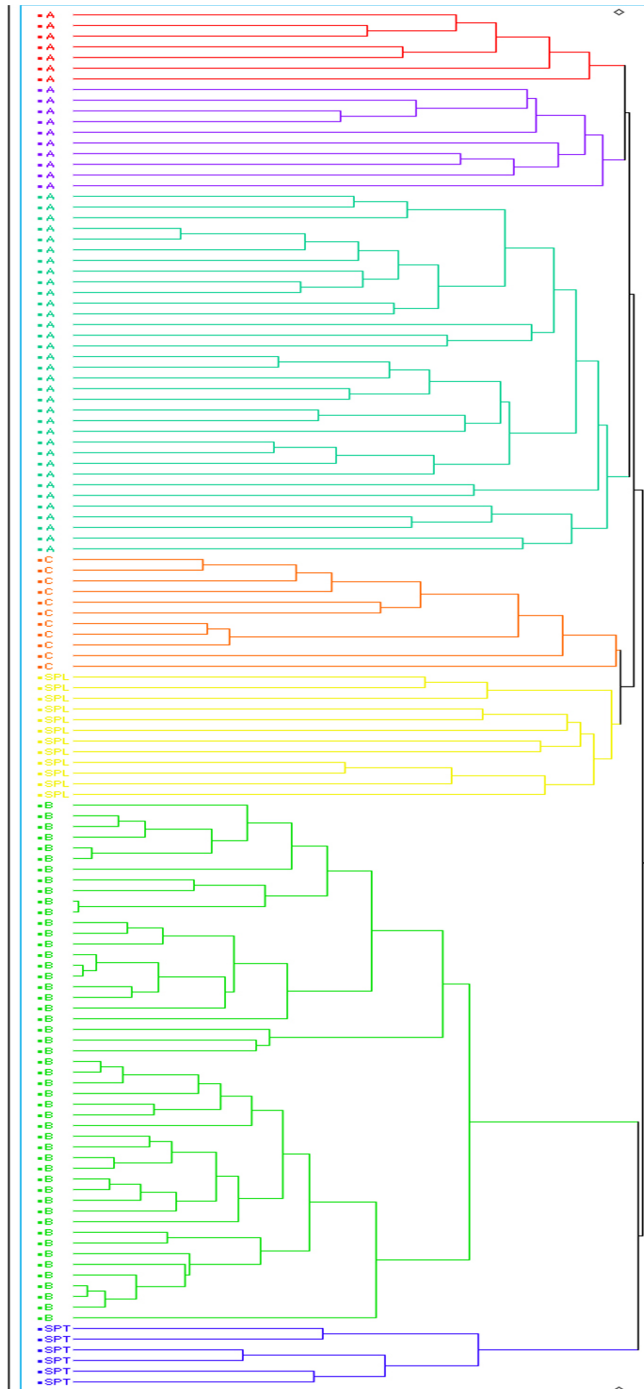


Hrusa Fig. 1. Principal Components Ordination. Axes 1 and 2. Colors correspond to UPGMA clusters in Fig. 3

PCA, Axes 1 and 3 (X & Z)  
 Salsola common garden plants  
 Quantitative plus qualitative chars.  
 12-2003



Hrusa Fig. 2. Principal Components Ordination. Axes 1(x) and 3 (z). Colors correspond to UPGMA clusters in Fig. 3.



Hrusa Fig. 3. California *Salsola*. UPGMA cluster analysis of common garden replicates. Classification as determined by isozyme profiles, performed by Dr. Fred Ryan, USDA. Branch colors correspond to PCA groups in Figs. 1 and 2.



Hrusa Figure 4. Fruit wings of *Salsola tragus* type A. Scale: numbered increments are 1 mm in length. Photo by G.F. Hrusa.



Hrusa Figure 5. Fruit wings of *Salsola* sp. type B. Scale: numbered increments are 1 mm in length. Photo by G.F. Hrusa.

## DISCUSSION

The complete isolation of type B from type A corroborates the earlier molecular analyses. The undisputed distinct species *Salsola paulsenii* ‘typical’ is even less separated from *S. tragus* type A than is the formerly included type B. and indicates that these are not “cryptic” taxa (see Ryan & Ayres op.cit.), but rather are completely distinct, in our opinion, full species. Characteristics separating Type A from Type B are given in Table 2; Figures 4 and 5 illustrate the minor (smallest of the five) fruit wing differences. Not all of the characteristics in Table 2 were used in this analysis, but they have proven useful in the field. These two types are highly recognizable once one knows what to focus upon, and can generally be distinguished while driving along at (no more than) the speed limit. Of biological interest are the adaptations that appear responsible for some of the differences between type A and type B. Early observations suggested that type A was tumbleweed, but that type B was not. Moreover, this distinguishing feature seems correlated with method of seed dispersal; type A winged fruits are found only near the summit of the plant, with unwinged fruits toward the base. Winged fruits are a clear adaptation to wind dispersal, and in type A, winged and unwinged fruits do not dehisce from the plant readily when mature. In contrast, fruits in type B are all winged and these disperse readily from the plant while it is still standing in place. Thus tumbling in type A appears to be a seed dispersal adaptation that is not utilized in type B; rather, type B uses its large calyx wings to disperse in the wind from a rooted plant. The fruits of type A are generally strongly clustered, although a few unwinged fruits may occur at basal internodes, most of the fruits (including wings) are longer than the internode at which they occur. In contrast type B winged fruits are consistently shorter than the subtending internode, despite the fruit wing being broader than in type A (see Table 2). This again seems an adaptation which allows type B fruits to fall easily from the plant. One can identify the two species when pressed in newspaper by the pile of loose separated fruits in type B; this never occurs in type A specimens.

HRUSA TABLE 2: Diagnostic characteristics of *Salsola* sp. type B vs. *Salsola tragus* type A.

Characteristic	Type A	Type B
Fruit diameter including wing	3.0 – 5.6 mm mean = 4.2 mm	5.7 – 7.9 mm mean = 6.9 mm
Minor wing shape	Linear	obovate
Winged fruit position on stem	upper ½ of plant	throughout plant
Anther length	0.6 – 1.3 mm mean=1.02 mm	0.45 – 0.7 mm. mean=0.58 mm.
Stem vestiture	gen. hairy, to +/-glabrous	glabrous
Fruiting internode length	shorter than adjacent bract	gen. longer than adjacent bract
Mature fruit behavior	persistent on plant	deciduous at maturity
Plant lifespan	Annual	annual to short-lived perennial
Plant shape	wider than tall	taller than wide
Post-senescent behavior	Tumbleweed	+/- persistent in place

## TWO POTENTIALLY SERIOUS NEW WEEDS IN CALIFORNIA

G.F. HRUSA

BOTANY LABORATORY/HERBARIUM CDA

### TWO POTENTIALLY SERIOUS NEW WEEDS IN CALIFORNIA.

Poaceae (Grass Family)

***Brachypodium sylvaticum* (Huds.) Beauv.** (Slender false-brome) Hrusa Fig. 1.

**Distribution in Ca.:** Central Coast

**Current Status:** naturalized

**Documentation:** San Mateo Co.: Perennial, in large colony beneath shade of coast redwoods. Hwy 84 at Grandview Terrace and Schilling Lake. Dec. 1, 2003, K. Melo s.n. (CDA and to be distributed)

**Discussion:** Native to central and northern Europe, this caespitose perennial grass is highly invasive in shady, moist conditions. In California it is known from a single extended infestation of approx. one (1) square mile centering around Schilling Lake, with additional scattered colonies from the east base of the Santa Cruz mountains up the Martin Creek drainage to near the crest at Sky Londa; a single outlier population is on the west slope approximately one mile distant. It occupies the understory of coast redwoods, coast live oak, Douglas-fir and other coastal tree and shrub species. It does not seem to grow in the deepest shade of redwoods but forms dense carpets where there are breaks in the canopy. It grows well under oaks and thrives in bright, somewhat moist areas. It is a non-rhizomatous bunch grass and, according to J. Johnson, (San Francisco Watershed Council), a colony can expand 2 or 3 feet a year under favorable conditions. It is also invasive in cultivated situations where there is an open understory. It is most aggressive in the openings of forests, but can also grow and reproduce in dense shade. It is unknown when or how this plant was introduced, but the infestation is centered on the former Schilling estate where there were extensive introductions of ornamental plants before the turn of the century. However, it may have been introduced inadvertently from Oregon more recently. Current molecular markers are being sought by a graduate student at Portland State University which may help determine its ultimate source.

Introduced also in northwestern Oregon as an ornamental in the 1930s, it remained localized until recently, but within the past 10-15 years has spread to occupy more than 10,000 acres. It is now found scattered across much of the Willamette Valley, in full sun to almost full shade, along logging roads and fire lines in coniferous forests.

Currently in both Oregon and California local task forces have been established with the goals of delimiting the infestations and potentially limiting their spread. Impacts are primarily ecological but the ultimate modification of the vegetation physiognomy, flora and fauna is currently unknown; the species forms near solid stands in areas where otherwise there was only an open mixture of often uncommon dicot herbs and few grasses, or there was no vegetative understory at all.





Hrusa Fig. 1. *Brachypodium sylvaticum* in the understory of coast redwood forest (*Sequoia sempervirens*), San Mateo County, California. Photo by J. Beall, San Mateo Co.



Rubiaceae (Madder Family)

***Diodia virginiana* L.** (Virginia buttonweed). Hrusa Fig. 2.

**Distribution in CA:** northern Central Valley

**Current Status:** naturalized in cultivated, agricultural situations.

**Documentation:** Shasta Co.: Lawn weed on Leonard St. Redding T31N, R05W Sec 23.. Sept. 18, 2003, *E. Finley s.n.* (CDA).

**Discussion:** This plant is a prostrate to semi-upright perennial that roots readily at the nodes and tolerates close mowing. It spreads both vegetatively and sexually, with self-fertilized flowers sometimes forming underground along the base of the rooted stems. Infesting sidewalk cracks and numerous lawns and gardens in the neighborhood above; the original source of the infestation is not known. This weed is a serious pest of turf in the southeastern U.S. where it has proven particularly difficult to eradicate (Uva et al. "Weeds of the Northeast" Cornell Univ. Press. 1997), however it does not appear to tolerate disturbance or cultivation and thus is not a common weed of field crops or gardens. It should be of interest to turf growers and landscape professionals that this plant not be allowed to spread beyond the location above, and particularly that it be excluded from commercial situations.



Hrusa Fig 2. *Diodia virginiana* (Virginia buttonweed). Sidewalk crack in Redding. Photo: E. Finley, (CDFA, IPC).

# Seed Laboratory

Staff:

Jim Effenberger

Don Joley

Deborah Meyer

Paul Peterson

Marian Stephenson

Julia Sher (USDA)

Elaine Harris

Evelyn Ramos

Connie Weiner

Jaime Sallee

Matt Wolfe

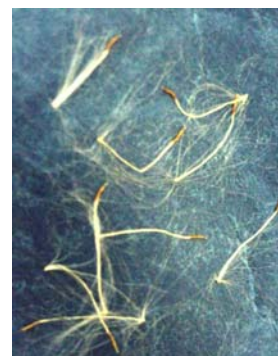
## Seed Herbarium Development

The PPDC Seed Collection is an essential tool for seed botanists who identify unknown fruit and seed specimens. The laboratory has a collection of more than 58,000 seed and fruit specimens, which is the second largest collection in the United States of this kind. Because California state is so diverse in every aspect of business and culture, new species from all points of the globe are brought into the state every year. In addition, the increasingly common practice of offshore seed production increases the likelihood of weed species from other parts of the world contaminating seed lots brought into the state. To help with this never-ending challenge the laboratory staff continually adds to the collection through garden, greenhouse, and windowsill cultivation of plants, as well as collecting seeds in the wild. Contributors to the CDFA Seed Herbarium this year include: Fred Hrusa, Evelyn Ramos, Marian Stephenson, and Connie Weiner.



WWW.Geocities.com

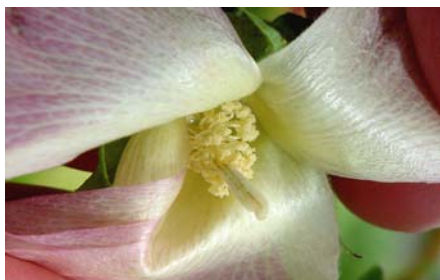
*Bromeliaceae*  
*Vriesia*



*Balsaminaceae*  
*Impatiens pallida*  
Pale touch me not



R. Mohlenbrock USDA-NRCS Plant



*Malvaceae*  
*Gossypium* sp.  
Cotton



*Lamiaceae*  
*Teucrium*  
*hircanicum*



Thompson & Morgan Ltd.

## Seed Technologist Training

Jim Effenberger, Elaine Harris, Deborah Meyer, Paul Peterson, Evelyn Ramos,  
Marian Stephenson and Connie Weiner

The responsibility of a seed technologist is to determine the quality of seed lots in commerce. Laboratory analyses serve as the basis for seed trade and thus the exchange of millions of dollars in seed sales globally. Standardization of laboratory test procedures is key to the success of the seed industry. With the goal of promoting standardization among seed laboratories, providing training via workshops and supervision of individualized training programs in the field of seed technology is one of the missions of the CDFA Seed Laboratory. Many individuals that have received training from the CDFA Seed Laboratory staff have become Registered Seed Technologists (RSTs) following passage of a nationally administered examination.

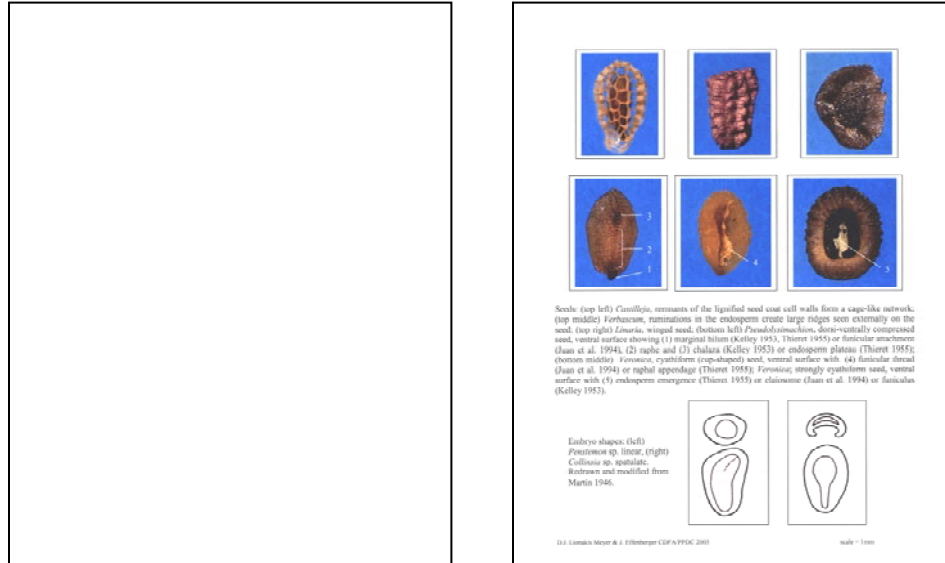
This year Jim Effenberger coordinated the California Seed Analysts and Seed Researchers 2003 Spring Workshop held at the CDFA Plant Pest Diagnostics Center, Sacramento, California. The laboratory technical staff was involved in preparation of hands-on materials for workshop participants to examine. The Seed Laboratory staff made the following presentations:

- Paul Peterson, Senior Seed Botanist – Commonly asked questions on vegetable and herb germination and seedling evaluation in the following families: Asteraceae, Apiaceae, Liliaceae, Brassicaceae, Chenopodiaceae, Cucurbitaceae, Fabaceae, Solanaceae, Tetragoniaceae.
- Deborah Meyer, Senior Seed Botanist - Identification of fruits and seeds in the Malvaceae (mallow family) and Scrophulariaceae (snapdragon family).
- Dr. Marian Stephenson, Senior Seed Botanist – Evaluation of seedlings in the Ranunculaceae (buttercup family).
- Jim Effenberger and Marian Stephenson, Senior Seed Botanists – Cotton seed quality assessment: seed structure, purity analysis, viability and seedling vigor.

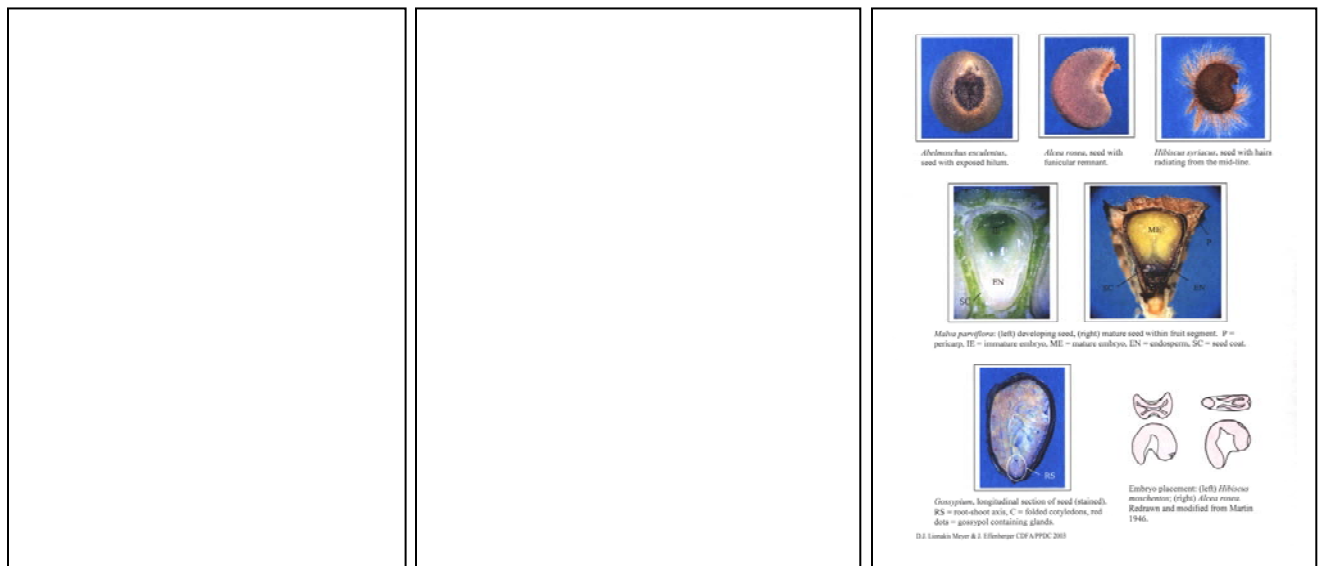


The *Cotton Seed Quality*  
workshop manual  
includes 38 color  
photographs detailing  
seed coat, embryo and  
seedling structure;





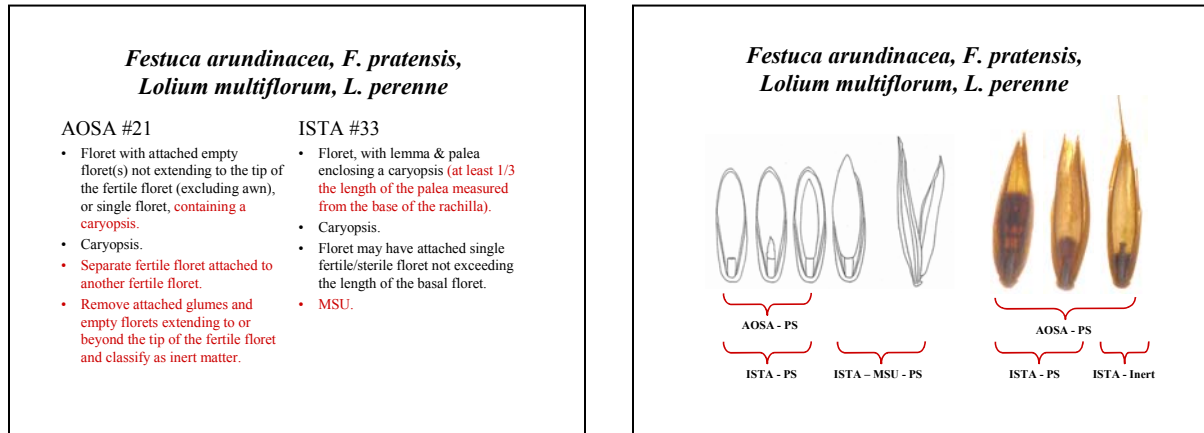
The *Scrophulariaceae Seed Identification* workshop manual contains general information about the snapdragon family, 94 color photographs of family and seed characters for 40 crop and weed species common in seed commerce.



The *Malvaceae Fruit Seed Identification* workshop manual contains general information about the mallow family, 153 color photographs of family, fruit and seed characters for 35 crop and weed species common in seed commerce.

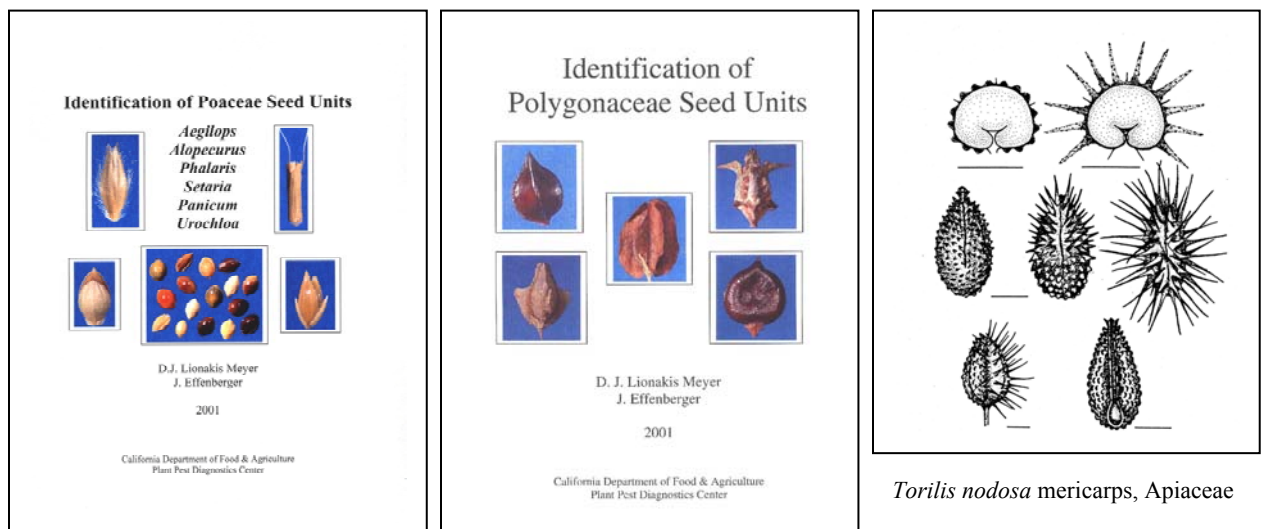
Deborah Meyer was an invited speaker at the International Seed Testing Association (ISTA) Purity Testing Workshop, held in Seattle, WA, June 10 – 12, 2003. Ms. Meyer's presentation was on the "Comparison of AOSA and ISTA Grass Seed Purity Testing Methods." The purpose of the presentation was to identify the similarities and

differences among the officially recognized laboratory testing procedures of the Association of Official Seed Analysts (AOSA) and the ISTA and to discuss the impact these differences may have on international seed trade. Laboratory testing procedures differ in well over half of the 79 grass species common to both organizations.



Example of the differences between AOSA and ISTA seed quality assessment procedures of certain lawn grass species, with substantial differences indicated in red. Example was taken from the presentation given at the 2003 ISTA Seed Purity Testing Workshop, Seattle, Washington.

Ms. Meyer served as an instructor at the Mid-West Seed Services 8<sup>th</sup> Annual Seed Analyst Training Workshop, Brookings, South Dakota, May 12 –13, 2003. Workshop participants were from independent, industry and government laboratories throughout the mid-western United States, Florida and Canada. Topics covered by Ms. Meyer included: seed development, structure and taxonomy of angiosperms (flowering plants); grass seed/fruit and associated structures and identification of *Aegilops* (goatgrasses), *Setaria* (bristlegrasses), *Panicum* (panicgrasses), *Urochloa* (millet), *Alopecurus* (foxtail grasses) and the wheatgrasses (*Agropyron*, *Elymus*, *Leymus*, *Elytrigia*); seed/fruit structures and identification in the Asteraceae (sunflower family), Apiaceae (carrot family), Polygonaceae (knotweed family).



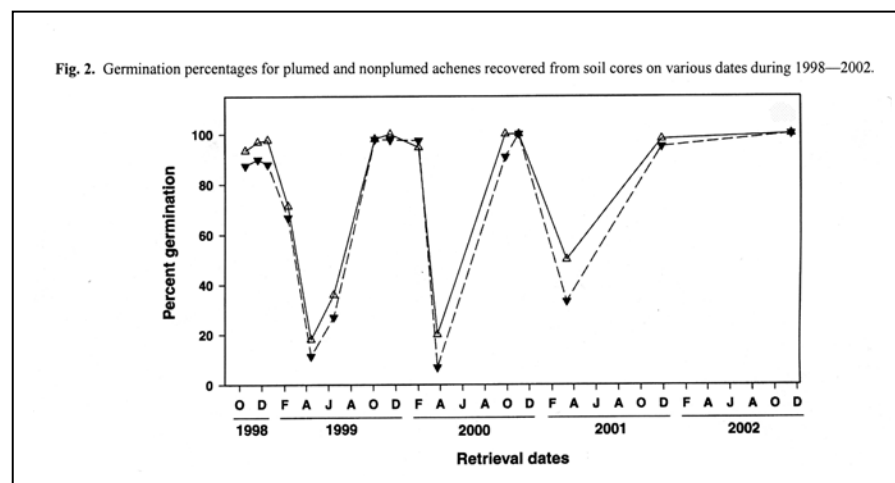
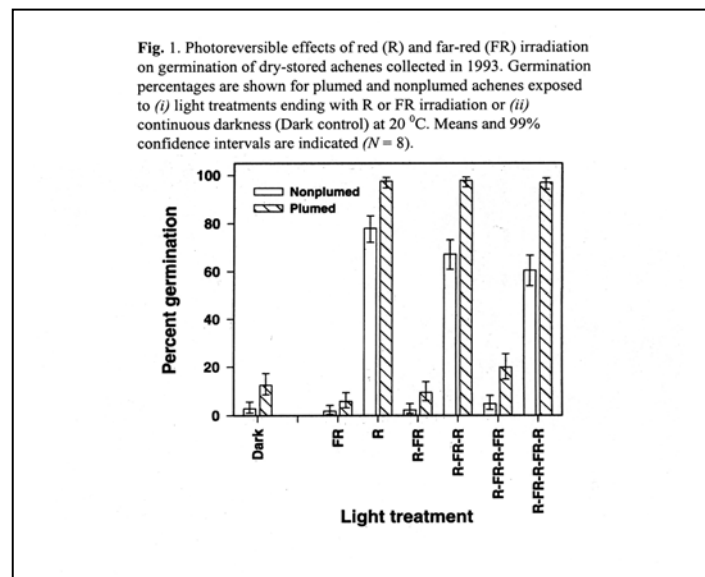
The *Identification of Poaceae Seed Units* workshop manual contains general information about the grass family, diagnostic keys and descriptions and 127 color photographs of seed unit characters for 37 crop and weed species common in seed commerce. The *Identification of Polygonaceae Seed Units* manual contains general information about the knotweed family, a diagnostic key, character tables and 77 color photographs for 25 crop and weed species common in seed commerce. The *Seed Unit Identification in the Apiaceae (Umbelliferae)* workshop manual contains general information about the carrot family, a diagnostic key to the fruits of 20 common crop and weed species and 75 original drawings by Ms. Meyer of the fruits of these species.



# Parameters affecting germinability and seed bank dynamics in dimorphic achenes of *Centaurea solstitialis* in California.

D.B. Joley, D.M. Maddox, S.E. Schoenig, and B.E. Mackey. 2003.  
*Can. J. Bot.* 81: 993-1007.

Yellow starthistle (*Centaurea solstitialis* L.) is a major rangeland weed pest in California and other western states. Two complementary aspects of seed biology were examined: germination and seed bank dynamics. Achenes were tested for changes in germinability over time using various light, temperature, and moisture treatments. Soil cores were collected over time to monitor changes in achene density. Both plumed and nonplumed achenes exhibited photo-reversible responses to red and far-red light (Fig 1). White and red light enhanced germination above that occurring in darkness. Achenes showed seasonal changes in germinability, which were characteristic of conditional dormancy. Exposure of achenes to field conditions during summer alleviated primary dormancy, allowing germination to occur at higher temperatures and lower substrate moisture. Nonplumed achenes collected in winter showed reduced germinability at or above moderate temperatures under both single and multiple light exposures. Imbibition and moderately low temperatures with subsequent drying induced secondary conditional dormancy in some achenes. Plumed achenes dominated the soil seed bank before the rainy season then declined rapidly following rain. Nonplumed achenes peaked after rains began then declined. Although nearly all achenes were depleted after 4 years, both types remained, showing annual cycles of high and low germinability (Fig 2) in autumn and late spring, respectively.



## ***Annual Report of Seed Laboratory Sample Workload 2003***

Jim Effenberger, Elaine Harris, Don Joley, Deborah Meyer, Paul Peterson,  
Evelyn Ramos, Marian Stephenson, and Connie Weiner

The staff of the Seed Laboratory of the Plant Pest Diagnostics Branch consists of five Seed Botanists, three Laboratory Assistants and additional support from temporary, part-time Scientific Aides. In the spring of 2003 the Seed Laboratory hired a new Seed Botanist to replace a recently retired staff member. During 2003, 83% of the workload consisted of seed quality assessment testing and seed/fruit identification, 7% was devoted to laboratory quality assurance (i.e., equipment maintenance and calibration, database management, Q.A. system development, seed herbarium curation) and 10% was devoted to professional enhancement activities (i.e., research, professional meeting attendance, workshop and seminar presentations, professional organization committee work, etc.).

### **Types of Samples Processed by the Seed Laboratory**

The Seed Laboratory routinely handles categories of samples as described below. Table 1 indicates the numbers of samples processed and tests completed during 2003 for each sample type. The percentages of tests completed for each sample type are shown in Effenberger et al. Figure 1.

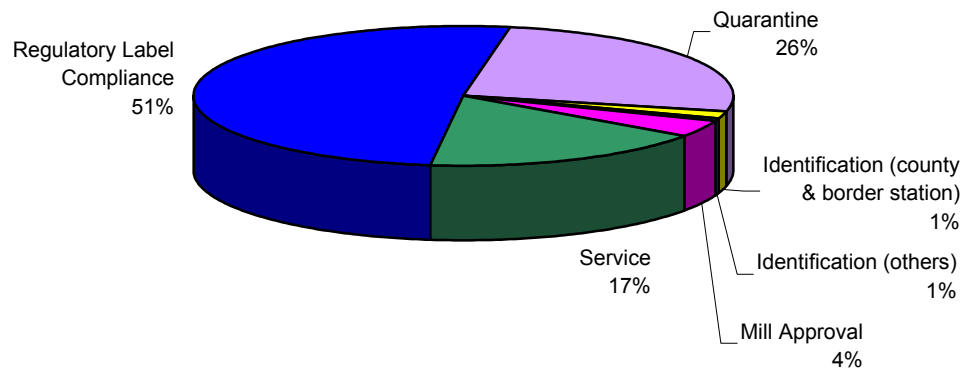
- **Quarantine** – Tests on quarantine samples require examination of a minimum of 25,000 seed units from each submitted sample to detect the presence of noxious weed seeds. Quarantine samples are drawn from seed lots moving across state and county lines and are an important part of the pest exclusion, detection and eradication program.
- **Regulatory** -Tests on regulatory label compliance samples include a noxious weed seed examination of a minimum of 25,000 seed units, a purity examination of a minimum of 2,500 seed units, and a germination test of 400 pure crop seed, from each submitted sample to determine label integrity. Laboratory procedures used for these tests are those prescribed in the Federal Seed Act. The noxious weed seed examination is similar to that of a quarantine test. The purity examination determines the physical composition of a seed sample and consists of separation of the pure crop seed kind or kinds (in the case of mixtures of 2 or more species) under consideration from the following contaminants: inert matter, other crop seeds, and weed seeds. The components are reported as percentages based on weight, and all contaminating species are identified. The germination test estimates the percentage of normal seedlings a seed lot can produce. Four hundred seed units are planted on various types of artificial media, and are subjected to various environmental conditions deemed appropriate for the species being tested, in an effort to determine the number of normal seedlings produced under optimum conditions. Laboratory results from the noxious weed seed examination, purity examination, and germination test are compared to the seed lot label; if the results are determined to be out of tolerance with the seed lot label, appropriate action is taken by Nursery and Seed Service. The percentages of the types of regulatory samples released to the Seed Laboratory in 2003 are shown in Effenberger et al. Figure 2.

- **Service** – Tests on service samples include examinations similar to those described for regulatory tests, as well as specialized tests based on client needs. Service samples are processed on a fee for service basis. The test results are reported directly to the client on formal certificates of analysis and are confidential. These documents are the basis for seed commerce throughout the world. Laboratory procedures used in service testing follow those prescribed in the Federal Seed Act, the Association of Official Seed Analysts Rules for Testing Seed, the International Seed Testing Association Rules for Seed Testing, and the Canadian Methods and Procedures for Testing Seed. Results of these tests may also be used for resolving contractual disputes. The percentages of the types of crops submitted as service samples are shown in Figure 3.
- **Feed Mill Approval** - Feed mill approval tests include the removal, identification, and determination of viability of all weed seed found in processed livestock feed samples. Testing of these samples regulates the certification of feed mills and stops the spread of weed seed throughout the state.
- **Identification** - These samples include identifications of specimens submitted to the laboratory by border stations, counties, other government agencies, commercial seed laboratories, medical doctors, veterinarians, archaeologists, and other researchers. These identifications are not only critical in preventing the spread of hazardous weeds, but are often necessary for expediting importation and exportation of agricultural products, are required as evidence in criminal court cases, and are necessary for medical and veterinary diagnoses of poisoning cases.

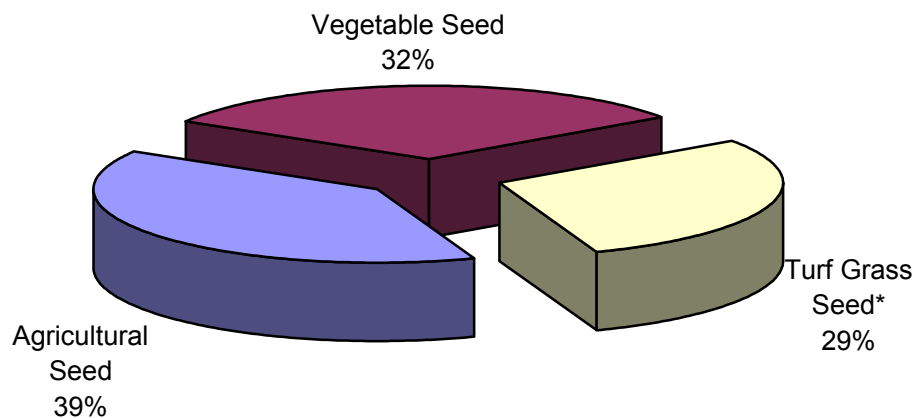
**Effenberger et al. Table 1.** Total number of samples processed and tests completed by the Seed Laboratory in 2003 for each sample type. Each sample received by the Seed Lab may require more than one test, with the type of test(s) dependent on the sample type.

Type of Sample	# Samples completed	# Tests completed
Quarantine noxious	1433	1433
Identification (county & border station)	58	78
Identification (others)	13	37
Mill Approval	76	210
Service	439	958
Regulatory label compliance	1048	2805
<b>TOTALS</b>	<b>3067</b>	<b>5521</b>

**Effenberger et al. Figure 1.** The percentages of tests completed by the Seed Laboratory in 2003 for each sample type. Pie areas represent percentages of the numbers of samples completed, not the time required to complete each type of sample.



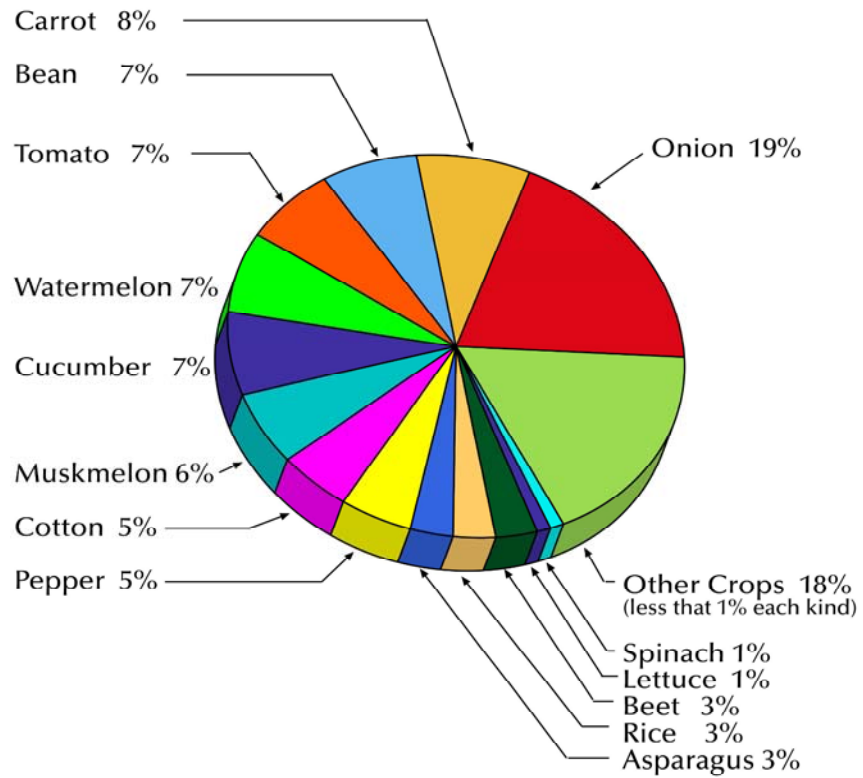
**Effenberger et al. Figure 2.** Percentages of the generalized crop types of regulatory samples released to the Seed Laboratory in 2003.



\* 41% of turf grass samples contained ryegrass, requiring fluorescence tests

\* 55% of turf grass samples were mixtures of 2 or more kinds of seeds requiring purity separation and separate germination tests.

**Effenberger et al. Figure 3.** Percentages of the types of crops submitted as service samples.



## A TAXONOMIC KEY TO THE FEDERAL NOXIOUS WEED SEEDS

Julia Sher, USDA

Federal noxious weed (FNW) disseminules (“seeds”) are prohibited from entering the U.S. or being transported in interstate commerce. USDA Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ) inspectors detect and intercept seeds from imported commodities at U.S. ports of entry nationwide, and PPQ identifiers ascertain whether these are FNW and thus actionable. Likewise, PPDC seed botanists examine samples of seed lots offered for sale in California to determine if any FNW are present.

Resources with which to identify the species listed as federally noxious (7 C.F.R. 360) are insufficient. A unique federal-state collaboration was established between USDA/APHIS/PPQ, Center for Plant Health Science and Technology (CPHST) and CDFA PPDC to rectify this problem. An employee was hired by CPHST in September 2002 to work at the Meadowview site with seed lab personnel to create a taxonomic key to the FNW seeds. The key will be created using Lucid software. Lucid keys are easy to use computer-based multi-access keys. Users can select characters to examine and are thus not hampered by the structure of a traditional paper-based dichotomous key. Identification is facilitated by multimedia (images, video, sound) attached to taxa and characters. Once identified, users may view detailed information about and images of a particular taxon in the form of “fact sheets” (Sher Figure 1).

CDFA’s extensive seed collection, library, catalog of images, and the expertise of seed lab botanists, are reasons why CPHST chose to locate the project here. It is hoped that the Lucid key to FNW seeds will be widely distributed, and a valuable identification tool for both partners in this collaboration.

**Drymaria arenarioides** Humb. & Bonpl. ex Schultes

**Caryophyllaceae**

**Common names** lightningweed, sandwort drymary, alfombrilla

Disseminule Seed

### Description

Fruit a capsule, 15-25 seeded. Seeds C-shaped, somewhat laterally compressed, with ends touching and slightly overlapping, 0.4-1.2 mm long  $\times$  0.4-0.8 mm wide  $\times$  0.3-0.6 mm thick, with a broad dorsal groove and sides deeply depressed. Seeds may assume atypical shapes due to crowding. Surface dull, light brown to brown, completely covered with minute puzzle-shaped tubercles. Hilum concealed on ventral surface. Embryo perisperm, curved around perisperm, reaching nearly to both ends. Cotyledons narrow. Perisperm semi-translucent, conspicuously starchy.



seeds



seeds



seed surface reticululation

## Identification remarks

Seeds in the genus *Drymaria* vary considerably with respect to seed shape, color, and surface sculpturing. Sculpturing may be merely granular to very ornate, with tubercles of various shapes and outlines. Attention to these characters can aid in species determination. Seeds in the genus *Silene* generally have flat lateral sides, with tubercles in regular rows that arise from variously shaped plates.

### Distribution

Mexico

**Habitat**

Dry areas, acid soils, hills and plains, stressed rangelands.

### General information

*Drymaria arenarioides* is a spreading perennial herb, up to 20 cm long. It invades rangeland, displacing desirable vegetation. Saponins in this plant are highly toxic to cattle, sheep and goats, resulting in major cattle losses in Mexico. While the plant is not documented in the U.S., it is spreading northward, reportedly to within one mile of New Mexico.



fruit

Sher Figure 1. Fact sheet for the federal noxious weed *Drymaria arenarioides*. This fact sheet will appear when the user of the Lucid key clicks on the name of the species within the multi-access key matrix. Photo by Julia Sher.

# Nematology Laboratory

Staff:

John Chitambar

Robert Hackney

Ke Dong

Rene Luna

Monica Negrette

Mirasol Ballesteros



## ANNUAL REPORT OF THE NEMATOLOGY LABORATORY'S SAMPLE PROCESSING AND NEMATODE DIAGNOSTIC OPERATIONS

John Chitambar, Ke Dong, Robert Hackney and Rene Luna

The Nematology Laboratory analyzed a total of 4,782 samples in 2003. Detail of sample distribution to various subprograms is given below:

<b>Quarantine Samples</b>	Total:	2,758
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Import:

External Quarantine Survey Burrowing and Reniform Nematode samples		2,394
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Border Station Interceptions		11
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Port Interceptions		1
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Export:

Phytosanitary Certification samples		343
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Other Quarantine samples		9
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<b>Nursery Samples</b>	Total:	1,997
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Nematode Control samples		542
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Garlic Certification samples		474
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Strawberry Certification samples		938
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Regulatory samples		8
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Other Nursery Certification samples		35
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<b>Commercial and Residential Samples</b>	Total:	4
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<b>Other Invertebrate Zoology/Parasitology Samples</b>	Total:	23
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## Nematodes of Special Interest Identified in 2003

### The Burrowing Nematode (*Radopholus similis*, Thorne 1949).

Females, males and larvae of this migratory endoparasitic nematode species were extracted from roots of ornamental palm (*Chamaedorea cataractarum* Mart.). The quarantine plants were introduced from Florida, intercepted twice in San Diego by County Agricultural inspectors and diagnosed and confirmed by the State Nematology Laboratory (Pest and Damage Record nos. 1260621 & 1260624). *Radopholus similis* is an A rated nematode pest requiring regulatory action.

### The Reniform Nematode (*Rotylenchulus reniformis*, Linford & Oliviera, 1940)

Fourth-stage larvae of this sedentary semiendoparasitic nematode species were extracted from roots of Devil's Tongue plants (*Sansevieria* sp. Thunb.). The quarantine plants were introduced from Florida, detected by Orange County Pathologist and confirmed by the State Nematology Laboratory (Pest and Damage Record no. 1254817). The nematode pest was rated Q as diagnosis was based on the detection of larvae only; however, the species closely resembled the A-rated species, *Rotylenchulus reniformis*.

### Chamber's Dagger Nematode (*Xiphinema chambersi*, Thorne, 1939)

Females and larvae of this ectoparasitic nematode species were extracted from soil collected around roots of an unknown plant that was intercepted by Border Station officials at Needles Inspection Station. The vehicle carrying the plant was entering California from Arizona. *Xiphinema chambersi* is a Q rated pest. The economic impact of the damage caused by *X. chambersi* remains unknown, and the nematode species has not been detected in California agricultural production sites. However, the damage potential of the genus-group *Xiphinema* is well documented and of sufficient economic importance to warrant quarantine status to *X. chambersi*. (Pest and Damage Record no. 1250629)

### The Spiral Nematode (*Helicotylenchus multicinctus*, (Cobb, 1893) Golden, 1956)

Females, males and larvae of this unusual nematode species were extracted from Banana roots and rhizomes (*Musa acuminata* Colla). *Helicotylenchus multicinctus* is an important root parasite of Banana worldwide. Unlike other species of the genus, *H. multicinctus* invades the cortical root tissue of Banana, and all life stages of the nematode may be found within root tissue as well as in rhizosphere soil. There is no evidence that the nematode is able to migrate through the root (like the Burrowing Nematode). Due to its endoparasitic feeding behavior, *H. multicinctus* can easily be introduced to virgin land through infested Banana soil and plant propagative material. The nematode species was detected by San Diego Pathologist and identified by the State Nematology Laboratory (Pest and Damage Record no. 1260011). There is no State record that indicates the earlier detection and established presence of *H. multicinctus* in California. The nematode species has never been rated specifically, but belongs to the genus-group *Helicotylenchus* spp. with a D rating that requires no regulatory action.

Given the documented damage potential and unknown occurrence of the nematode species in California, it is evident that *H. multicinctus* needs to be treated as a quarantine pest with an A rating.

#### The Root Lesion Nematode (*Pratylenchus* spp.)

Five species of *Pratylenchus* were detected during 2003, namely: *P. brachyurus* (30 detections), *P. coffeae* (3 detections), *P. penetrans* (8 detections), *P. vulnus* (14 detections) and *P. zae* (1 detection). With the exception of *P. coffeae*, all the other species are found more or less commonly in California agricultural production sites. All species of the genus are of economic importance with significant damage potential to agricultural crops. Although historically, *P. coffeae* has been detected by the State Laboratory in few instances in California, it has not been detected in agricultural production sites. In 2003, *P. coffeae* was detected in roots of ornamental plants, *Aglaonema* sp., and *Ficus benjamina* imported from Florida and intercepted by San Diego and San Bernardino County Agricultural officials. *Pratylenchus coffeae* is a C rated pest, however, given the damage potential and apparent absence of this species from California agricultural soils, it is likely that this pest warrants a higher rating requiring quarantine action.

## Pinewood Nematode Species Complex

Ke Dong

The pine wood nematodes, *Bursaphelenchus xylophilus* and *B. mucronatus*, are members of the pinewood nematode species complex (PWNSC). Biological studies showed that these two nematodes shared strong similarities in many respects – morphologically, vector relationships, life cycle, and host trees.

*B. xylophilus* is native to North America. It was found in association with fungi in timber in 1929 and described as *Aphelenchoides xylophilus* (Steiner and Buhrer, 1934). Nickle (1970) transferred this nematode to *Bursaphelenchus* (JON 2:375-392). Mamiya and Kiyohara (1972, *Nematologica* 18:120-124) reported *Bursaphelenchus lignicolus* (n. sp.) in Japan but this species was placed as a synonym of *B. xylophilus* (Nickle, 1981, JON 13: 385-392). It was believed that the nematode was introduced from North America to Japan around the turn of the century. Molecular data also supported the theory that Japanese and American strains of *B. xylophilus* might be derived from common origins. It was first demonstrated to be a pathogen on pine trees in 1971 (Kiyohara and Tokushige). The wilting caused by *B. xylophilus* in susceptible pines (Asian species) results in serious economic and environmental damage to Chinese and Japanese pine forests. Because this disease is regarded as a serious threat to European forests, the European Community (EC) has instituted a ban on the import of coniferous timber which has not been kiln dried that originates from regions where *B. xylophilus* is endemic. In the USA, however, *B. xylophilus* has not caused any epidemic wilting disease in American pine species. In addition, the nematode has not caused the death of American pine species planted in China or Japan, suggesting that North American pine species may have resistance to this nematode. *Bursaphelenchus mucronatus* (Mamiya and Enda, 1979, *Nematologica* 25: 353-361) is widely distributed in Eurasia, from most of the European countries to Japan. *B. mucronatus* has been reported in Canada, but has not been found in the United States. In general, *B. mucronatus* has been reported as a weak pathogen. *B. mucronatus* is often found in dead or dying pine trees but not known to initiate disease in nature in non-stressed trees. Inoculation tests showed that the mortality caused by *B. mucronatus* was low (generally 4.2%-6.2%), which was not much greater than non-inoculated controls. Under the same testing conditions, however, *B. xylophilus* can cause greater than 64 percent tree mortality. No typical wilting symptoms in pine trees infected by this nematode are reported in nature in either Chinese or Japanese publications (Mamiya and Enda, 1979, *Nematologica* 25: 353-361. Cheng et. al. 1986. *Journal of Nanjing Agric. Univ.* 1986: 55-61).

*B. mucronatus* is reported to have a broader distribution than *B. xylophilus* in Asia. Biological studies have shown that these two species can be isolated from the same infected hosts, including dead trees, and they share the same insect vectors in nature. ‘Pure’ cultures can be established under lab conditions by selecting and inoculating with single adult females. The two species are differentiated by the tail shape (Cheng et. al. 1986). *B. mucronatus* differs morphologically from *B. xylophilus* only by the presence and length of a mucro ( $>3.5\ \mu\text{m}$ ) on the tail terminus. Certain isolates of *B. xylophilus* are also reported to have mucronate tail but usually the mucrones are not longer than  $2\ \mu\text{m}$ . Similarly, in North America, morphotypes of *B. xylophilus* with some

characteristics of *B. mucronatus* have been recovered in the US and Canada. They are called “M” forms (mucronate form) as compared to the typical “R” form (round tail). Molecular techniques have been conducted to differentiate the two species and certain DNA fragments may have value in determining taxonomic affinities (Harmey and Harmey, 1993, JON25: 406-415). ITS sequences also support the perception that *B. mucronatus* group and *B. xylophilus* are distinct genetic entities (Becknbach et. al., 1999, *Nematology* 1: 539-548). Based on the molecular data (Harmey and Harmey, 1993, JON25: 406-415. Becknbach et. al., 1992. JON24: 140-147) there exist two *distinct B. mucronatus* groups: one group containing the European isolates, and another containing the Japanese isolates. In addition, tremendous DNA-based variations are found among the isolates of PWNSC, at both species and strain levels.

Differences among virulent and avirulent isolates of *B. xylophilus* have been reported in Japan and in the US. In general, the “R” form isolates showed greater virulence to tested pine species, e.g. *Pinus sylvestris*, *P. strobus*, *P. nigra*, *P. taeda* and *P. thunbergii*. However there were also some avirulent “R” forms, or the pine species showed strong tolerance to these isolates (Bolla and Boschert, 1993. JON 25: 227-238). Among the “M” forms, a Japanese isolate was highly virulent to the tested pine species, but the other isolates were moderately virulent (Bolla and Boschert, 1993). An “M” form of *B. xylophilus* was reported to be highly virulent on *Pinus massoniana* in China (Cheng, 1986). “M” forms also have been recovered from dead or dying conifers in France, Norway and Siberia. A French “M” form isolate of *B. xylophilus* which was never found in healthy trees, but found in declining *Pinus pinaster* trees that were not necessarily under stress, were more pathogenic than *B. mucronatus*, but not as pathogenic as *B. xylophilus* (de Guiran and Boulbria, 1985. *Nematologica* 35: 321-330). There were two *B. mucronatus* isolates tested in Bolla’s report (1993), both of which were avirulent to the tested pine species.

The biological species concept is based on the definition of interbreeding. The validity of *B. mucronatus* was contested by Baujard (1980), but confirmed by Nickle et al (1981) on the basis of its reproductive isolation from *B. xylophilus* in mating experiments (Yashuaru Mamiya’s test being mentioned in Nickle’s report, JON 13: 385-392). However, inter-specific hybridization has been demonstrated between *B. mucronatus* and *B. xylophilus* in many later publications. de Guiran and Bruguier (1989) reported that a French “M” form isolate could produce normal fertile hybrids with *B. mucronatus* (a Japanese isolate) as well as with *B. xylophilus* (also a Japanese isolate), but the same isolate did not produce fertile hybrids when mated with an “M” form isolate from Minnesota (*Nematologica* 35:321-330). Riga et al. (1992) conducted an interspecific and intraspecific cross hybridization test in which the male nematodes from a Japanese *B. mucronatus* isolate mated successfully with females of four different isolates of *B. xylophilus*, suggesting that the two species came from a common ancestor (*Fundam. Appl. Nematol.* 15: 391-395). Bolla and Boschert (1993) demonstrated that interbreeding occurred in the laboratory between some “M” and “R” forms of *B. xylophilus*, and the hybrids exhibited the pathogenicity of the parent with the broader host range. Interbreeding of *B. xylophilus* and *B. mucronatus* was rare but did occur. They suggested

that virulence might be inherited as a dominant character or that increased virulence may have resulted from differences in hybrid vigor (*JON* 25: 227-238).

Having reviewed the available information from the published literature, *B. mucronatus* is not known to occur in the US. It is a weak pathogen to some pine species, but inter- and intra-specific variations among isolates of nematodes are observed, and the nematode strain/pine host specific interactions are not thoroughly understood. Although the nematode is not known to be a killer of pine trees, the relationship of the decline and growth reduction of pines and the infection by the nematode is not clear. Susceptibility of US pine species under natural conditions in North America is unknown. Variations in susceptibility may exist at the strain, variety, sub-species, and species levels.

Interbreeding with *B. xylophilus* has been demonstrated. The hybrids may have the pathogenicity of the parent with the broader host range, and interbreeding of virulent isolates with avirulent isolates might lead to production of new, highly virulent hybrids. Morphological identification is not always reliable. Dwinell and Nickle considered “M” form isolates to be closely related to *B. mucronatus*, but molecular data indicated that the “M” forms were within the *B. xylophilus* group. Unequivocal identification of these nematodes is further complicated by the inter-fertility of *B. mucronatus* and *B. xylophilus*. Many nematologists preferred to use the terms such as the pine wood nematode species complex (PWNSC) or supra-species to address this group of nematodes.

DIAGNOSTICS OF MELOIDOGYNE CHITWOODI GOLDEN, O'BANNON, SANTO &  
FINLEY, 1980 FOR REGULATORY ACTION

Robert W. Hackney

September 2003 the United States Department of Agriculture (USDA) accepted the California Department of Food and Agriculture's (CDFA) diagnostic protocol (viz. APPENDIX I) for the regulatory diagnostics of Columbia Root Knot Nematode (CRKN), *Meloidogyne chitwoodi*, infesting potato tubers for export to Mexico. CDFA's Nematology Laboratory/Program began developing this diagnostic protocol in 1979, which was prior to the published description of the *M. chitwoodi*.

The fundamental problem was potatoes from the western United States, infested with CRKN, were arriving in Mexico. Mexican authorities inspected the potatoes, performed their diagnostics for CRKN using polymerase chain reaction (PCR) based techniques and rejected infested shipments. The USDA wanted a reliable diagnostic protocol to inspect potatoes for CRKN, which would pass thorough scrutiny. The CDFA/Nematology protocol was provided as a benchmark for the USDA, other diagnostic facilities and the Mexican government.

**APPENDIX I**

**<sup>1</sup>Regulatory Diagnostics: *Meloidogyne chitwoodi* Infesting Potato Tubers**

1. An aliquot of any sample of tubers submitted for nematode diagnostics shall consist of those tubers exhibiting symptoms and/or signs of RKN plus additional randomly selected tubers to total 200.
2. The Nematology Laboratory shall recover female RKN from all sample aliquots of 200 tubers each as follows:
  - a. Each tuber is gently washed with tap water known not to already contain any of the nematodes associated with the current diagnostics.
  - b. Clean tubers shall be externally examined for symptoms and/or signs of RKN.
  - c. Tubers shall be sliced to reveal at least 10 thin (i.e., 1-1.5 mm) slices representing a selective bias toward any external symptoms and/or signs.
    - i. The 10 slices shall reflect a collection of cross sections, tangential sections and sagittal sections passing through an axis from each end of the tuber.
    - ii. Tuber slices shall be examined using either natural sunlight or halogen illumination to reveal RKN infection site(s). Those

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<sup>1</sup> Robert W. Hackney, Ph.D., Senior Plant Nematologist, California Department of Food and Agriculture, Plant Health and Pest Prevention Services, Plant Pest Diagnostics Center, 3294 Meadowview Road, Sacramento, California 95832-1448, USA. Phone: (916) 262-1115, FAX: (916) 262-1190, E-mail: <mailto:rhackney@cdfa.ca.gov>, Web Site: <http://www.cdfa.ca.gov/phpps/ppd/Nematology/NemaIndexPage.htm>

infection site(s) appear as translucent infested areas in normally opaque healthy tuber tissue.

- d. Infested tubers shall be dissected to retrieve intact female RKN, which are to be identified as RKN by a qualified Nematologist and held in saline buffer solution adjusted specifically for RKN.
  - i. The Nematologist shall either produce a diagnostic taxon/taxa report (DTR) of RKN to the Latin mononominal (i.e., Genus), if possible and/or subject the collection of females held in saline buffer to further tests (i.e., enzyme, PCR and possibly cytogenetics) for further identification to the Latin binomial (i.e., Genus and species).
3. If necessary, a morphological examination of female RKN held in saline buffer shall be conducted by a qualified Nematologist, who examines perineal patterns using the oil immersion objectives (i.e., 50/54/97/100 X) on a compound light microscope equipped with Differential Interference Contrast (DIC) optics and pertinent filters.
  - a. The Nematologist shall either produce a diagnostic taxon/taxa report (DTR) of RKN to the Latin mononominal (i.e., Genus), if present and/or subject the collection of females held in saline buffer to further tests (i.e., enzyme, PCR and possibly cytogenetics) for further identification to the Latin binomial (i.e., Genus and species).
4. If necessary, a biochemical evaluation of female RKN held in saline buffer shall be conducted to study their enzyme phenotypes, as needed, and to provide a pertinent differentially diagnostic supplement (i.e., esterase [Est], catalase [Cat], glutamate-oxaloacetate transaminase [Got], glycerol-3-phosphate dehydrogenase [Gpd]  $\alpha$ -glycerophosphate dehydrogenase and/or malate dehydrogenase/superoxide dismutase [Mdh/Sod], superoxide dismutase [Sod]).
5. If necessary, an evaluation of female RKN held in saline buffer shall be conducted using the Polymerase Chain Reaction (PCR) technique/technology by selecting the appropriate differentially diagnostic pairs of primers to provide a pertinent differentially diagnostic supplement.
6. If necessary to further resolve an apparent mixed field population of species of RKN, the cytogenetics of females held in saline buffer shall be conducted by determining the diploid (2N) and/or haploid (N) chromosome number by staining the chromosomes with propionic-orcein stain and direct observation with a compound light DIC microscope using oil immersion optics.



# Entomology Laboratory

## Staff:

Fred Andrews (emeritus)  
Charles Bellamy  
Thomas Eichlin (retired)  
Marc Epstein  
Eric Fisher  
Stephen Gaimari  
Raymond Gill  
Alan Hardy  
Terry Seenro  
Ron Somerby  
John Sorensen

Scott Kinnee  
Ramona Randolph  
Mary-Jean Sawyer  
Joanne Virone  
Jenny Chau  
Matt Fossum  
Peter Kerr  
Randy Plant  
Joe Posades  
Ernie Riberal  
Jo Viray  
Kevin Williams

# SYSTEMATICS OF THE BUPRESTOIDEA LEACH, 1815 (COLEOPTERA): PROGRESS REPORT 2002-2003

C. L. Bellamy

The 2002 progress report was omitted from the lab's 2002 annual report, so I have combined that with data from 2003.

## Introduction

The superfamily Buprestoidea contains two families: the small southwestern North American endemic Schizopodidae LeConte, 1859 (2 tribes, 3 genera, 6 species) and the eighth largest beetle family, the cosmopolitan Buprestidae Leach, 1815 (6 subfamilies, 46 tribes, 488 genera, 14,702 species). The faunas of these two families for America, north of Mexico have been summarized in two chapters of *American Beetles* (Bellamy & Nelson, 2002; Nelson & Bellamy, 2002). The Schizopodidae were last revised by Nelson & Bellamy (1991).



*Glyptoscelimorpha (Dystaxiella) juniperarum* Knull  
on juniper in eastern San Diego County



A mating pair of *Dystaxia elegans* Fall  
on live oak in eastern San Diego County

The Buprestidae are such a large family that specialization, either in lower ranking taxa or regional faunas, is necessary. A general, popular article on the family was recently published:

Bellamy, C. L. 2003. The Stunning World of Jewel Beetles. *Wings*. Essays on Invertebrate Conservation, Fall 2003, pp. 13-17.

My specialization has been in several different taxa or regional projects which are summarized on the PPDB web site:

[www.cdfa.ca.gov/phpps/ppd/Entomology/Coleoptera/Buprestidae/index.html](http://www.cdfa.ca.gov/phpps/ppd/Entomology/Coleoptera/Buprestidae/index.html)

Many of these projects are detailed on the website with the URLs listed under each heading below.

## 1. The Madagascan Coraebini

[www.cdfa.ca.gov/phpps/ppd/Entomology/Coleoptera/Buprestidae/MadCor/intro.html](http://www.cdfa.ca.gov/phpps/ppd/Entomology/Coleoptera/Buprestidae/MadCor/intro.html)

The tribe Coraebini Bedel, 1921, is the largest tribe in the family (ca. 150 genera) and currently underway is the large descriptive work for the vast undescribed fauna of Madagascar (see Bellamy, 2001), where an estimated 500-600 new species will need 20-30 new genera to be added to the known fauna of 29 genera and 104 species. Much of the undescribed

material was collected from nest-provisioning wasps of the genus *Cerceris* and resulted in many thousand specimens made available to my study in the Museum National d'Histoire Naturelle, Paris. I was awarded an Ernst Mayr grant from the Museum of Comparative Zoology, Harvard University in 2003 to travel to the Paris Museum to continue this work.

The following publication described three new genera and seven new species:

Bellamy, C. L. 2003. The Madagascan Coraebini (Coleoptera: Buprestidae: Agrilinae): Part 4, New genera and species. *Zootaxa* **174**: 1-19.

## 2. The Buprestidae of Mexico

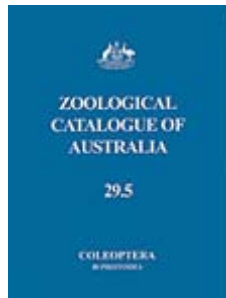
[www.cdfa.ca.gov/phpps/ppd/Entomology/Coleoptera/Buprestidae/Mexico/index.html](http://www.cdfa.ca.gov/phpps/ppd/Entomology/Coleoptera/Buprestidae/Mexico/index.html)

Bellamy C. L. & H. A. Hespenheide. 2002. The review of two Mexican species-groups of *Agrilus*. (Coleoptera: Buprestidae: Agrilinae). *Anales del Instituto de Biología, Universidad Nacional Autónoma de México, Serie Zoología* **73**(1): 37-51.

## 3. The Buprestidae of Australia

[www.cdfa.ca.gov/phpps/ppd/Entomology/Coleoptera/Buprestidae/AussieCat/austcat.html](http://www.cdfa.ca.gov/phpps/ppd/Entomology/Coleoptera/Buprestidae/AussieCat/austcat.html)

This area of interest has resulted in a new catalogue and a recent paper:



Bellamy, C. L. 2002. Coleoptera: Buprestoidea. In: Houston, K. W. W. (Ed.). *Zoological Catalogue of Australia*, Volume 29.5 Buprestoidea. CSIRO Publishing, Melbourne, Australia, xii + 498 pp., 4 color plates.

Williams, G. & C. L. Bellamy. 2002. New species and new records for the genus *Nascioides* Kerremans, 1903 (Insecta: Coleoptera: Buprestidae). *Zootaxa* **58**: 1-9.

## 4. The Tribe Haplostethini LeConte, 1861 has resulted in a recent paper:

Bellamy, C. L. 2002. The *Mastogenius* Solier, 1849 of North America (Coleoptera: Buprestidae: Polycestinae: Haplostethini). *Zootaxa* **110**: 1-12.

## 5. The World Catalogue of Buprestoidea

[www.cdfa.ca.gov/phpps/ppd/Entomology/Coleoptera/Buprestidae/WorldCat/intro.html](http://www.cdfa.ca.gov/phpps/ppd/Entomology/Coleoptera/Buprestidae/WorldCat/intro.html)

The page-formatted catalogue files currently stand at 3018 pages and it was essentially completed at the end of 2003. The effort to complete this catalogue has resulted in the following publications:

- Bellamy, C. L. 2002. Case 3193. *Chrysodema* Laporte & Gory, 1835 and *Iridotaenia* Deyrolle, 1864 (Insecta, Coleoptera): proposed conservation of usage by the designation of *C. sonnerati* Laporte & Gory, 1835 as the type species of *Chrysodema*. *Bulletin of Zoological Nomenclature* **59**(3):185-187.
- Bellamy, C. L. 2003. Case 3194. *Lius* Deyrolle, 1865 (Insecta, Coleoptera): proposed conservation. *Bulletin of Zoological Nomenclature* **60**(2): 132-133.
- Bellamy, C. L. 2003. Nomenclatural and taxonomic changes, new distribution and biological records for jewel beetles (Coleoptera: Buprestidae). *Insecta Mundi* **16**(1-3): 57-63.
- Bellamy, C. L. & M. G. Volkovitsh. 2003. Case 3258. *Acmaeodera* Eschscholtz, 1829 and *Acmaeoderella* Cobos, 1955 (Insecta, Coleoptera): proposed conservation. *Bulletin of Zoological Nomenclature* **60**(1): 31-33.
- Bellamy, C. L. & R. L. Westcott. 2003. Correction to the Buprestidae and Schizopodidae listings from *Nomina Insecta Nearctica* (1996). *Zootaxa* **147**: 1-20.
- Bellamy, C. L. & R. L. Westcott. 2003. Case 3257. *Acmaeodera oaxacae* Fisher, 1949 and *Polycesta deserticola* Barr, 1974 (Insecta, Coleoptera): proposed precedence of the specific names over those of *Acmaeodera philippinensis* Obenberger, 1923 and *Polycesta aruensis* Obenberger, 1924 respectively. *Bulletin of Zoological Nomenclature* **60**(2): 124-126.
- Bílý, S. & C. L. Bellamy. 2002. Case 3205. *Cyphosoma* Mannerheim, 1837 (Insecta, Coleoptera): proposed conservation, and *Halecia* Laporte & Gory, 1837 (Insecta, Coleoptera): proposed precedence over *Pristiptera* Dejean, 1833. *Bulletin of Zoological Nomenclature* **59**(4): 249-252.
- Davidson, J. M. & C. L. Bellamy. 2002. The entomological contributions of Josef Nissley Knull (1891-1975). *Zootaxa* **37**: 1-24.
- International Commission of Zoological Nomenclature. 2002. Opinion 2008 (Case 3149). 30 species-group names originally published as junior primary homonyms in *Buprestis* Linnaeus, 1758 (Insecta, Coleoptera): conserved. *Bulletin of Zoological Nomenclature* **59**(3): 211-216 which was the result of: Bellamy, C. L. 2001. Case 3149. Proposed conservation of 31 species-group names originally published as junior primary homonyms in *Buprestis* Linnaeus, 1758 (Insecta, Coleoptera). *Bulletin of Zoological Nomenclature* **58**(1): 24-31.

The classification scheme planned for the world catalogue was tested with the recent volume:

- Bellamy, C. L. 2003. An illustrated summary of the higher classification of the superfamily Buprestoidea (Coleoptera). *Folia Heyrovskiana, Supplement* **10**, 197 pp., 44 color plates, 453 figures.



New taxa proposed during 2002-2003:

#### Family-group

*Eucallopistina* Bellamy, 2003, replacement name for *Callopistina* Kurosawa, 1990 (Asian subtribe)

*Mimicoclytrina* Bellamy, 2003, replacement name for *Acherusiina* Cobos, 1995 (Neotropical subtribe)

#### Genus-group

*Aaaba* Bellamy, 2002, replacement name for *Alcinous* Deyrolle, 1865, preoccupied) (Australia)

*Dejongiella* Bellamy, 2003, new genus for *Discoderes cavifrons* Fairmaire, 1904 (Madagascar)

*Eucallopistus* Bellamy, 2003, replacement name for *Callopistus* Deyrolle, 1865 (preoccupied) (Asia)

*Mimicoclytrina* Bellamy, 2003, replacement name for *Acherusia* Laporte & Gory, 1837 (preoccupied) (Neotropical)

*Neefia* Bellamy, 2003, new genus for seven new species (Madagascar)

*Neefioides* Bellamy, 2003, new genus for *Cisseis rufobasalis* Fairmaire, 1897 (Madagascar)

*Zitoides* Bellamy, 2003, replacement name for *Zita* Bellamy, 1992 (preoccupied)  
(Afrotropical)

Species-group

*Acmaeodera ruficaudis macfadyeni* Bellamy, 2003, replacement name for *A. ruficaudis pinguis* Holm, 1985 (preoccupied) (Afrotropical)

*Agrilus bicoloropsis* Bellamy & Hespenheide, 2002 (Mexico)

*Agrilus clytrinoides* Bellamy & Hespenheide, 2002 (Mexico)

*Dicerca reticulatoides* Bellamy, 2003, replacement name for *D. reticulata* Assmann, 1870 (preoccupied) (fossil)

*Mastogenius arizonicus* Bellamy, 2002 (Arizona)

*Mastogenius texanus* Bellamy, 2002 (Texas)

*Melobasis novaeguinae* Bellamy, 2003, replacement name for *M. papuana* Obenberger, 1938 (preoccupied) (New Guinea)

*Nascioides caledonicus* Williams & Bellamy, 2002 (first species of genus from New Caledonia)

*Nascioides subcostatus* Williams & Bellamy, 2002 (Australia)

*Neefia gracilis* Bellamy, 2003 (Madagascar)

*Neefia humeralis* Bellamy, 2003 (Madagascar)

*Neefia magna* Bellamy, 2003 (Madagascar)

*Neefia montana* Bellamy, 2003 (Madagascar)

*Neefia rufofascia* Bellamy, 2003 (Madagascar)

*Neefia rufovestita* Bellamy, 2003 (Madagascar)

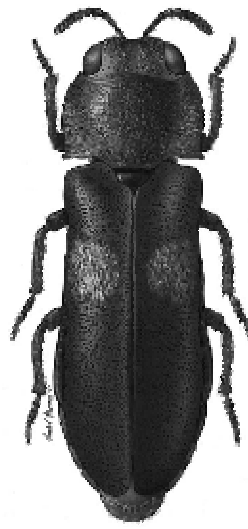
*Neefia semivestita* Bellamy, 2003 (Madagascar)



*Nascioides caledonicus*



*Mastogenius arizonicus*



*Agrilus clytrinoides*



*Neefia humeralis*

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- Bellamy, C. L. & G. H. Nelson. 2002. Chapter 41. Buprestidae Leach 1815, pp. 98-112. In: *American Beetles*, Volume 2: Polyphaga: Scarabaeoidea through Curculionoidea 2002.  
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- Nelson, G. H. & C. L. Bellamy. 2002. Chapter 40. Schizopodidae LeConte 1861, pp. 95-97. In: *American Beetles*, Volume 2: Polyphaga: Scarabaeoidea through Curculionoidea 2002.  
Editors: R. H. Arnett, Jr., M. C. Thomas, P. E. Skelley and J. H. Frank.



### **PPDB AND THE COLEOPTERISTS SOCIETY**

Terry Seeno and Chuck Bellamy, PPDB Entomology Insect Biosystematists served as officers for the Coleopterists Society in 2003. Terry completed his seventh year as the Society treasurer and Chuck completed the first of a two-year term as Society President.

Both attended the annual meetings of the Society (Executive and General Business meetings) held in conjunction with the larger annual meetings of the Entomological Society of America, in Cincinnati, Ohio, October 26-29 and the Entomological Collection Network meetings, October 25-26, 2003.

Terry's role as Society treasurer involves all aspects of financial (dues, invoices, investment management, tax return preparation) and membership records and transactions. He also serves as the Society webmaster: [www.coleopsoc.org](http://www.coleopsoc.org)

As President, Chuck presides and conducts both the Executive and General Business meetings and invites the speaker for the annual Tuesday evening General meeting during his term. This year the Coleopterist's Society inaugurated the new Honorary member award that acknowledged five long-time Society members '*recognizing devotion to the Society and dedication to the discipline*'.



## PPDB ENTOMOLOGISTS AND EDITORIAL RESPONSIBILITIES

Three PPDB insect biosystematists currently serve in a voluntary editorial capacity as follows:

Ron Somerby

Editor: *Pan Pacific Entomologist*, the journal of the Pacific Coast Entomological Society. Ron's involvement continues a long history of CDFA scientists and the editorial functions of this journal. Those who have previously served in this capacity include Fred Andrews, Bob Dowell, Tom Eichlin, Alan Hardy, Dick Penrose and John Sorenson.

Steve Gaimari

Publications Committee: *Pan Pacific Entomologist* (2001 – present)

Editorial Board: *Dipteron, Zeitschrift für Dipterologie* (1999 – present)

Diptera Subject Editor: *Annals of the Entomological Society of America* (2001 – present)

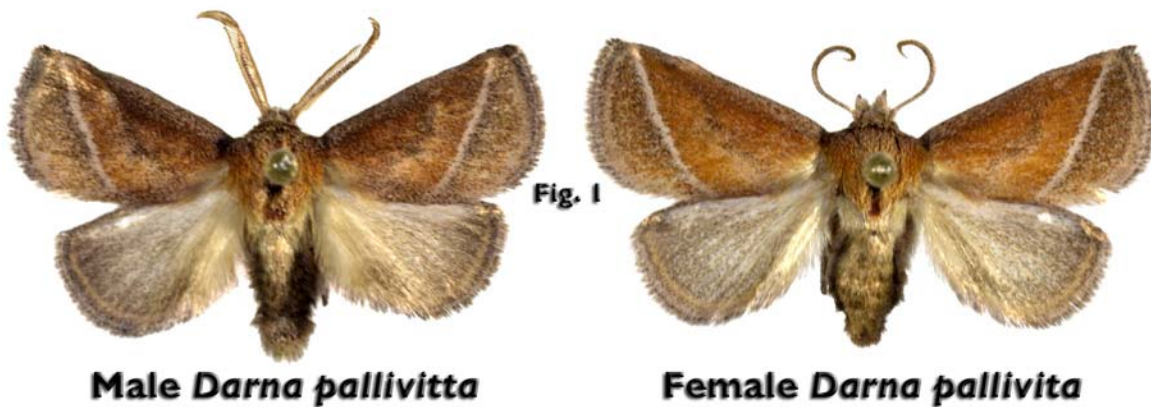
Chuck Bellamy

Coleoptera Subject Editor: *Zootaxa* (2001 – present)

English language editor: *Folia Heyrovskyana* (2002- present)

# On the lookout for *Darna pallivitta* Moore: A Recently Established Moth in Hawaii

Marc E. Epstein and Scott A. Kinnee



The moth family Limacodidae (approx. 1000 species), the slug and nettle caterpillars, occur worldwide primarily in the tropics. Presently only three species of around 50 North American species of limacodids are known to occur in California. Species in the related family Zygaenidae, such as the grape-leaf skeletonizer (*Harrisina brillians*), are better known to California agriculture.



Over the past few years two moth species in these two families have established in the United States, both of Asian origin. The limacodid is *Darna pallivitta* Moore and the zygaenid is *Pryeria sinica* Moore. *Darna pallivitta* will likely persist on the big island of Hawaii due to its broad range of larval hosts, typical of many Limacodidae. Epstein made the initial identification of this moth and has been an advisor on the rearing

colony and biological control of the species by the Hawaiian Department of Agriculture (HDA). *Pryeria sinica* has been found in both Maryland and Virginia (Brown et al. 2004), which as resulted in a recent pest alert by APHIS, PPQ. The larval feeding of *P. sinica* is restricted to the family Celastraceae. Large infestations of the larvae were reported on ornamental *Euonymus* in Fairfax, Virginia in April and May of 2001-2003.

Although it is important to be on the lookout for both of these species moving into California, this report will focus on *Darna pallivitta*. The specimens and the color images presented in this report were provided by Walter Nagamine of the HDA. A future publication describing its biology and a detailed description of its life history is planned in collaboration with Nagamine. The scanning electron micrographs (SEM) of the first two instars of the larva were recently produced by Kinnee at CDFA.



The larvae of limacodid moths are unusual in several respects. The name slug caterpillar comes from their unique undersurface, which has sticky sucker disks that move in smooth peristaltic waves. Caterpillars of limacodids and zygaenids have retractile heads, enabling them to feed while hiding the motion of the mouthparts (Epstein 1996).

The dorsal surface of these caterpillars can be quite variable, ranging from having spiny warts referred to as tubercles or scoli to being smooth or granulate. The former are referred to as nettle caterpillars, while the latter are the gelatine caterpillars. Limacodids often undergo major changes in the form of the dorsal surface between the first and second instars. This is particularly true of many smooth caterpillars, which lose their tubercles in the second instar.

*Darna pallivitta* was previously known as a minor pest from China, Taiwan, Thailand, W. Malaysia, Indonesia, and Java (Holloway et al. 1987). It was first discovered on Hawaii in 2001 by workers handling *Rhapis* palms, who had itching reactions to the stinging hairs of the larvae (Nagamine, pers. comm.). The larva has since been found on a number of monocots including *Dracaena* and several dicots.



The adults of *D. pallivitta* (Fig. 1) are typical limacodids, with stout bodies and bipectinate antenna in the males (forewing length: 9 mm) and threadlike antennae in the larger females (forewing length: 10-11 mm). They are unlike any other species reported from California (note: at present, the limacodid species found in California are known

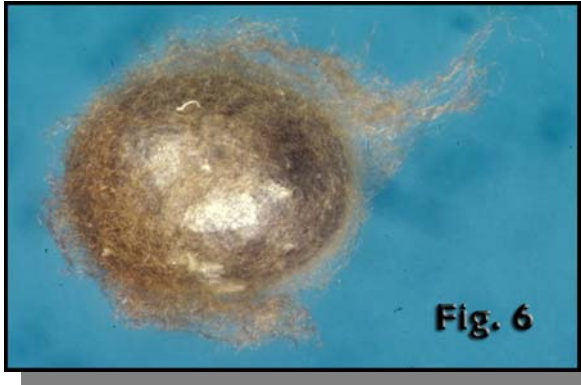


from the adult stage only). The forewing has a diagonal line that runs from near the tip to past the midpoint along the inner margin. In later instars the larva is gray with two rows of spines borne on scoli, with light stripe along the dorsal row of spines and along the median (Fig. 2). *Monoleuca*

*occidentalis* Barnes & McDunnough, is likely to be of the only California species to have a spiny, nettle type

larva that could be confused with *D. pallivitta*. *M. occidentalis* is known from Ventura Co. to Baja California. The first three or four instars feed on only one surface, whereas the later instars feed can either make holes through the leaves or feed on edges (Figs. 3-5). The cocoons are oval and hard (Fig. 6). On emergence the pupa pushes open a lid and is partially extruded before the adult climbs out.

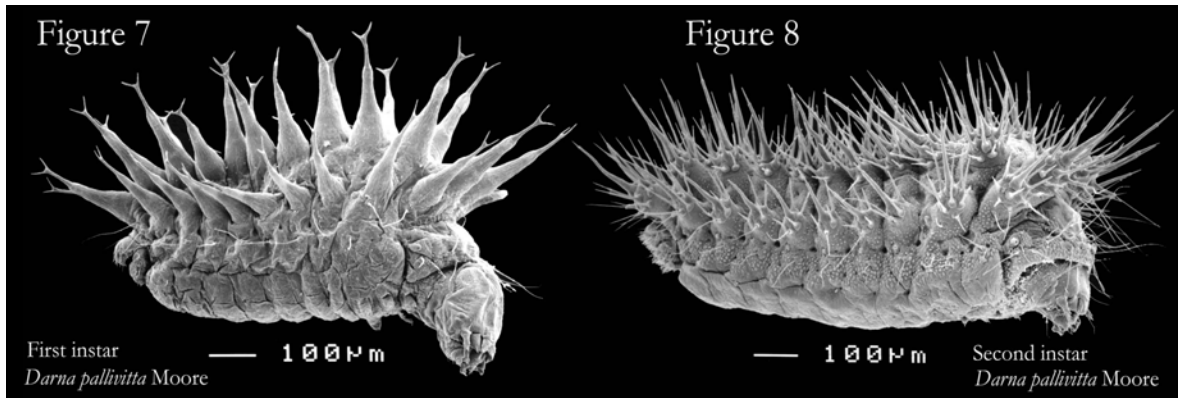
Last year there were at least two caterpillars and cocoons of *D. pallivitta* intercepted in California or on route to the state. One larva came on a Lei from Hilo,



Hawaii on 30 May 2003. It was intercepted by PPQ and sent to the National Museum of Natural History, Smithsonian Institution, where Epstein made the determination. The second larva was found on Illima flowers from Keaau, Hawaii on 31 July 2003. This specimen was sent to the Plant Pest Diagnostics Center, CDFA, by the Alameda County Agriculture Commission. The cocoons found on a

shipment of fishtail palms from Hilo, Hawaii on 22 Sept. 2003. The shipment was rejected by CDFA in Stockton. Coincidentally, the summer of 2003 had an unusually large number of limacodid caterpillars from Central America or Mexico reported on cut flowers in Europe (Epstein pers. comm.).

While the life history of *D. pallivitta* is reported in the literature (Holloway et al. 1987), there are no images or a detailed description of the early larval stages. SEM reveals that there is a large increase in the number of spines between the first and second instars that is typical of limacodids with spiny caterpillars (Figs. 7-8). It is believed that this change in form is due to being constrained to develop in a flat, scale-like egg with a very thin chorion (Epstein 1996). The first instar larva of *D. pallivitta* has some setal characters that have not been observed in other spiny limacodids thus far. The two-part branching at the apex of the tubercles has only been found in species that become spineless in later instars. Likewise, the presence of a hairlike L seta on the meso- and metathorax is also unique.



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## POSTDOC IN FRUIT FLY SYSTEMATICS

Eric Fisher, Steve Gaimari and Peter Kerr

A new dimension was added to PPDB in September 2003, with the arrival of Dr. Peter Kerr- our first Postdoctoral Fellow in Insect Systematics. This three-year position is funded by a grant from the USDA, and is a collaborative effort between the University of California, Davis (they supply the academic and administrative components) and the PPD Lab (we supply the working space and project oversight).

Peter will specialize on the systematics of Tephritidae (fruit flies) during his tenure at CDFA/UCD, and will emphasize the combination of molecular and morphological methodologies in investigations that are aimed at enhancing the identification process of various tephritid pest species that are important to California agriculture.

The major focal areas for this position are:

1. Perform original research on the systematics of Tephritidae, using a question-driven approach and utilizing relevant methodologies as necessary. These may include, for example, phylogenetic analyses of morphological character systems, molecular methods, biogeographical studies, and analyses of patterns of host utilization and phytophagy. Peter has selected a portion of the large American genus *Anastrepha* (which includes the Mexican fruit fly, *A. ludens*, as well as other important pest species) for this research; Allen Norrbom (SEL/ USNM) will collaborate in this study.
2. Development of molecular diagnostic tools for two main purposes (below), both of which will greatly aid in the accurate and timely diagnostics of potential invasive pests into California, and will help in developing diagnostics protocols for invasive fruit flies worldwide:
  - a. To expand the ability of making accurate species-level identifications of larval fruit flies;
  - b. To aid in demographic analyses of Mediterranean fruit fly (*Ceratitis capitata*), and possibly other key pest species, to foster identifications of specific origins and pathways of pest introduction into California.

Peter Kerr is a recent Ph.D. graduate of the University of Maryland, in a program associated with the U.S. National Museum of Natural History, although coming to work at the CDFA is somewhat of a homecoming for him. He grew up in Ventura and graduated with a B.A. in Biology at the University of California at Santa Cruz. One full year of his undergraduate studies was spent at the Universidad de Costa Rica in San Jose, where he studied tropical entomology under the tutelage of William Eberhard and Paul Hanson. After graduating, he worked as a research assistant at the UC Cooperative Extension in Ventura, but his interests in tropical biology led him to return to Latin America to collect insects and work as a guide in the Amazon Basin in Ecuador. For his doctoral thesis, Peter presented new morphological and molecular data in a phylogenetic framework to address longstanding problems of classification of the Rhagionidae and questions regarding the origins of Brachycera (Diptera). Peter is now looking forward to applying the expertise gained by combining his training in molecular techniques at the University of Maryland and fundamental studies of dipteran morphology at the Smithsonian Institution to address tephritid diagnostics, population biology, and systematics.



## CALIFORNIA STATE COLLECTION OF ARTHROPODS:

### 2003 PROGRESS REPORT

**Stephen D. Gaimari & Charles L. Bellamy**

The California State Collection of Arthropods (CSCA) is a scientific resource for the local, federal, and international community for research and identification of various groups of arthropods, especially insects. The collection is maintained by the Entomology Lab of the Plant Pest Diagnostics Branch of the California Department of Food and Agriculture, as an integral feature of the identification services provided to the citizens and business interests of the State, and to our peers and colleagues both nationally and internationally. Two curators (the authors) directly supervise the care, use, growth and development of CSCA, encouraging the use of this collection for research on the taxonomy and systematics of arthropod taxa. The web page for the collection is located at the following URL: <http://www.cdfa.ca.gov/phpps/ppd/CSCA.htm>.

The total number of prepared specimens exceeds 1.5 million, with more than 20,000 prepared specimens accessioned in 2003. One holotype and numerous paratypes were deposited in CSCA in 2003, and the collection is being recognized as an important repository for certain groups of arthropods. The databasing of the collection is in its early stages, with bar-code labels providing unique identifiers for each specimen.

Our status as the officially recognized State Collection paved the way for the California Department of Parks and Recreation to issue the scientists a blanket permit for collecting arthropods and plants in the entire State Park system.

As far as specimen usage, the CSCA issued 22 loans in 2003, representing more than 13,000 specimens, and more than 25 visitors from the local, national, and international communities have come in to study our collections. Additionally, several client groups have been given tours of the collection.



## RESEARCH ON FLIES (DIPTERA)

**Stephen D. Gaimari**

- A. Details were provided in the 2002 CDFA/PPD Annual Report for the following paper that was published in 2003:

Anderson, M.S., & S.D. Gaimari. 2003. Raman-Atomic Force Microscopy of the ommatidial surfaces of Dipteran compound eyes. *Journal of Structural Biology* 142: 364-368.

- B. In 2003, Steve contributed a chapter to the European Commission funded project, "Fauna Europaea," aimed at documenting the known fauna of Europe and providing distributional information by country for each of over 130,000 species. The website for this project is found at the following URL: <http://www.faunaeur.org/>. Steve's submitted chapter covers the 107 species of the predacious fly family Chamaemyiidae, which has known species in nearly all parts of Europe.

- C. The following papers, with a brief comment each, are *in press*, and will be published early in 2004.

1. Gaimari, S.D., L.S. Adler, & S.J. Scheffer. 2004. Plant host affiliation and redescription of *Phytomyza subtenella* Frost (Diptera: Agromyzidae). *Proceedings of the Entomological Society of Washington*.

The agromyzid genus *Phytomyza* contains more than 450 described species worldwide, all of which have internally feeding, plant-parasitic larvae. Most species are leafminers, but some are known to feed in other plant parts, including stems, roots, flowers, and seeds. Members of this genus attack species in nearly 30 plant families, although most individual *Phytomyza* species are highly restricted in diet, usually feeding on one to few closely related plant species. Within *Phytomyza*, morphologically similar species tend to feed on closely related plants (those in the same genus or family) in a pattern suggestive of host-associated radiations. Currently, in North America and Europe, there are 13 *Phytomyza* species known from Orobanchaceae, parasitic plants that obtain water and nutrients from other plants via root connections. Although phylogenetic relationships among these and other *Phytomyza* species have not been explored, genitalic similarities suggest that there are several distinct lineages within this group, with unknown affinities among these lineages. Here, we report the host plant for *Phytomyza subtenella*, having reared it from seeds of a hemiparasitic Indian paintbrush, *Castilleja miniata*. We also report observations on its natural history and provide a redescription and more detailed drawings of genitalia than were previously available in the literature. My thanks are extended to Fred Hrusa for lots of help with the issues surrounding the plants in this study.

2. Thunes, K.H., et alia (47 authors; S.D. Gaimari, author 34). 2004. The arthropod community of Scots pine (*Pinus sylvestris* L.) canopies in Norway. *Entomologica Fennica*.

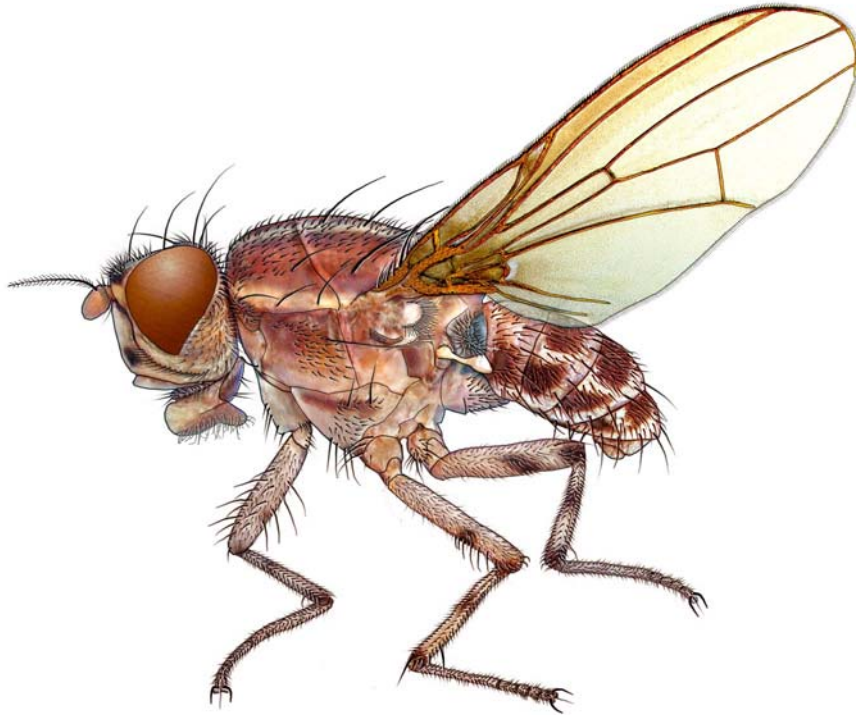
Steve contributed identifications for and information about Chamaemyiidae and Lauxaniidae in this effort to understand the arthropod community of a single species of pine in a high north latitude country (i.e., where you would expect low diversity), to show that even in such an environment, the biodiversity is enormous. Through fogging the canopies of 24 Scots pine trees with insecticide, nearly 30,000 specimens were collected and distributed to specialists worldwide. These were identified as more than 500 species, including more than 200 species of Diptera alone; 82 of the species were new records for Norway, 3 were new to Scandinavia, 2 were new to Europe, and 9 were species new to science.

3. Yang, D., & S.D. Gaimari. 2004. Discovery of *Systemus* in the Oriental Region, with description of one new species (Diptera: Dolichopodidae). *Pan-Pacific Entomologist*.

To date, 23 species of *Systemus* are known, with 8 species from the Palaearctic Region, 6 from the Nearctic, 7 from the Neotropical, and 2 from the Australian. This paper represents the first record of this genus from the Oriental Region, with the description of this new species. The larval habitats are known for at least half of the species in this genus, all of which are associated with (likely as predators within) humid rotting debris within tree-holes or sap exudates from tree wounds.

- D.** The following papers, with a brief comment each, have been *submitted*, and are currently undergoing review.

1. Gaimari, S.D. 2004. A new genus of Lauxaniidae (Diptera) from New Caldeonia. *Zootaxa*.



A new lauxaniid genus from New Caledonia (see figure above) is described and illustrated, along with six new species. Evolutionary relationships among the six species are hypothesized, character states are discussed, and a key to the genus and its species is provided. Although many other new species of other genera are recognized for New Caledonia, this paper more than doubles the described fauna of lauxaniids for the island.

2. Gao, C., D. Yang, & S.D. Gaimari. 2004. The subgenus *Euhomoneura* Malloch (Diptera: Lauxaniidae) in the Palaearctic Realm. *Pan-Pacific Entomologist*.

The genus *Homoneura* is the largest genus in the family Lauxaniidae, and is distributed worldwide except for the Neotropical Region, but its subgenus *Euhomoneura* was previously found only in the Oriental and Australasian Regions with few species. This paper represents the first record of this subgenus in the Palaearctic Region, with descriptions and illustrations for two species new to science.

3. Noma, T., M.J. Brewer, K.S. Pike, & S.D. Gaimari. 2004. Hymenopteran parasitoids and Dipteran predators of *Diuraphis noxia* (Mordvilko) (Hemiptera: Aphididae) in the west-central Great Plains: species list, new species records, and geographic range. *Proceedings of the Entomological Society of Washington*.

The focus of this paper is to list species of parasitoids and predatory flies attacking Russian wheat aphid (RWA) found in the west-central Great Plains of the United States, highlight new records of natural enemy species found in the region, and note their geographical ranges. Also species diversity was compared between this study (15 years after the first appearance of RWA in the region) and previous surveys (during and soon after the release of exotic natural enemies).

4. Yang, D., & S.D. Gaimari. 2004. Review of the species of *Elaphropeza* Macquart (Diptera: Empididae: Tachydromiinae) from the Chinese mainland. Proceedings of the Entomological Society of Washington.

The species of the empidid genus *Elaphropeza* from the Chinese mainland are reviewed, and two species new to science are described and illustrated. Also, a key to the 22 species of this genus from the Chinese Mainland is presented for the first time.

# Plant pathology Laboratory

Staff:

Cheryl Blomquist

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Julie Traina

## DETECTION OF *WISTERIA VEIN MOSAIC VIRUS* IN CALIFORNIA

Tongyan Tian<sup>1</sup>, Heather Scheck<sup>2</sup>, Deborah Mayhew<sup>1</sup>, and Julie Traina<sup>1</sup>

<sup>1</sup>Plant Pest Diagnostics Center, California Department of Food and Agriculture;

<sup>2</sup>Agricultural Commissioner's Office, Santa Barbara County.

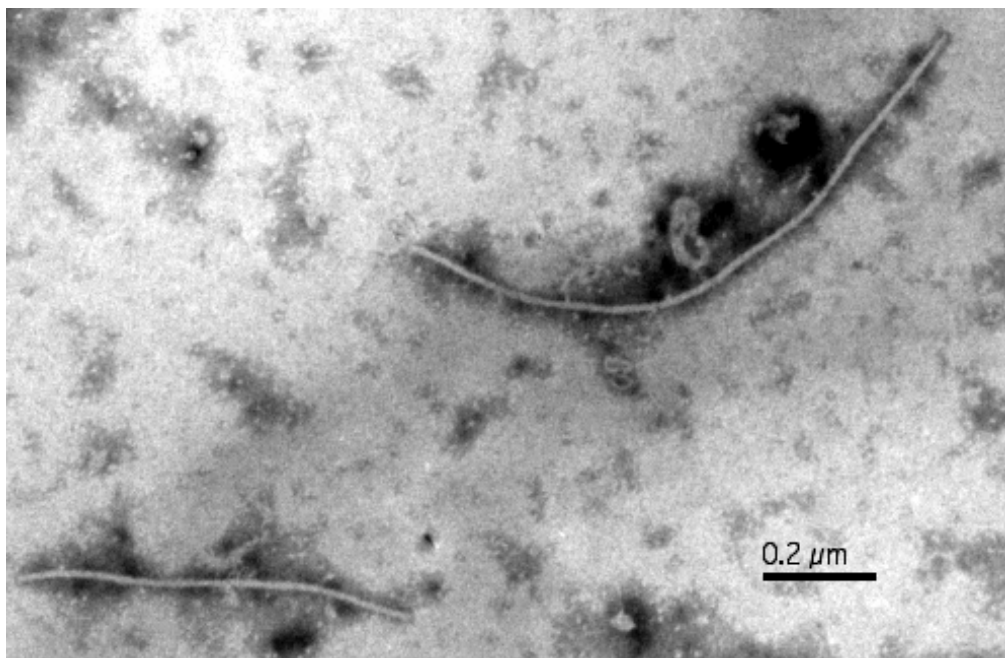
*Wisteria* (*Wisteria spp*) is a relatively common ornamental plant found throughout the world. As early as 1957, Brierley and Lorentz reported a possible virus disease on *Wisteria floribunda*. The symptoms included yellowish blotches of irregular size and shape. In 1970, Bos determined the virus disease on wisteria which exhibited similar symptoms was caused by *Wisteria vein mosaic virus* (WVMV). The determination was based on host range analysis, virus particle morphology and its serological relationship with other viruses.

Previously, wisteria plants showing typical yellow blotches on the leaves (See Figure 1) had been submitted to the Plant Pest Diagnostics Center for virus disease detection. We found flexuous rod-shaped virus particles from sap of the plants similar to virus particles illustrated in Figure 2. Because serological and molecular assays were not available for (WVMV), the disease was mistakenly determined as a disease caused by *Watermelon mosaic virus* (WMV) based on serological assays. Recently, however, questions were raised regarding the causal agent of the symptomatic wisteria in California, whether it was WVMV or WMV. Therefore, we investigated our diagnostic procedures on those wisteria plants.



Tian et al. Figure 1. Leaf symptoms of *Wisteria vein mosaic virus* (photo from Heather Scheck)

We investigated whether symptomatic wisteria plants were infected with WVMV or WMV. We used partial nucleotide sequence of WVMV published by Clover *et al.* 2003 and designed WVMV specific oligo-primers (Table 1) and also adapted oligo-primers from the same publication for diagnostics.



Tian et al. Figure 2. Image of potyvirus particles. Photo by T. Tian.

Table1. Oligo-primers for WVMV.

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<sup>a</sup> WVMV-R1A	5'-TGGATATTCAACCTGCTCTT-3'
<sup>a</sup> WVMV-F1A	5'-TGTGCAGCWATGATWGARGC-3'
<sup>b</sup> WVMV-R1	5'-CAGTATTCTC GACTGTTGTT GAGA-3'
<sup>b</sup> WVMV-F1	5'-AGTTTACTT GTGGTTGCTT GAA-3'
<sup>b</sup> WVMV-R2	5'-GGAACAACAA ACATTGCCGT ACCT-3'
<sup>b</sup> WVMV-F2	5'-AATGGAAGAG TGTGTGAGT CAGT-3'

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<sup>a</sup>Oligo-primers described by Clover et al. 2003.

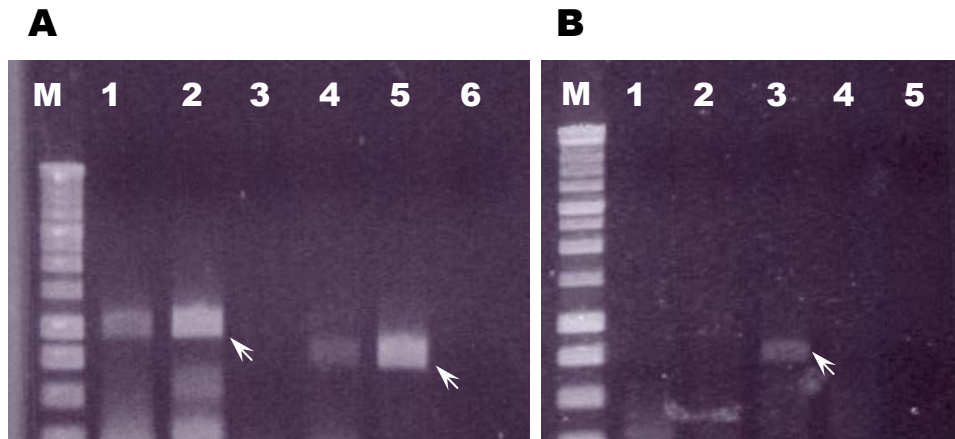
<sup>b</sup>Oligo-primers designed according to partial WVMV nucleotide sequence in the GenBank, accession number AF484549.

We used two wisteria samples that tested positive for WMV using ELISA and one wisteria sample that tested negative for WMV to start our investigation. Using total RNA extracted from symptomatic wisteria, we conducted reverse transcription polymerase chain reaction (RT-PCR) using oligo-primer pairs, WVMV-R1A and WVMV-F1A, WVMV-R1 and WVMV-F1, WVMV-R2 and WVMV-F2. Expected RT-PCR products were detected using agarose gel electrophoresis analysis (Figure 3A). We also compared



RT-PCR using total RNAs from symptomatic and asymptomatic wisteria plants, WMV infected and healthy cucumber plants. We were able to amplify RT-PCR products only from the symptomatic wisteria, but not from the asymptomatic wisteria and WMV infected and healthy cucumbers (Figure 3B). In contrast, we were able to amplify WMV specific RT-PCR product only from WVM infected cucumber using WMV specific oligo-primers (data not shown). These results clearly demonstrated that the wisteria plants were infected with WVMV, not WMV. We tested 13 additional wisteria samples for WVMV from two counties in California using RT-PCR. Seven plants were positive for WVMV.

WVMV is a member in the family of *Potyviridae* and is transmitted by aphids *Aphis craccivora*, *Myzus persicae*, in a non-persistent manner (Brunt *et al.* 1996). WVMV is closely related to WMV, *Soybean mosaic virus* (SMV), and *Bean common mosaic virus* (BCMV) (Clover *et al.* 2003). Cross-reactions have been observed for WVMV in ELISA using antisera against WMV and BCMV (Clover *et al.* 2003; Elmhirst and Edwards 1998). These data and our own observation indicate that previous determination of WMV in wisteria is likely due to cross-reaction.



Tian *et al.* Figure 3. RT-PCR products from RNAs extracted from wisteria and cucumber plants. **A**: RT-PCR using primer set WVMV-R1 and WVMV-F1 (lanes 1 and 2), and primer set WVMV-R1A and WVMV-F1A (lanes 4 and 5). Arrows point to RT-PCR product for WVMV. **B**: RT-PCR using primer set WVMV-R1A and WVMV-F1A. M, 1kb DNA markers (Promega); 1, cucumber infected with WMV; 2, healthy cucumber; 3, a symptomatic wisteria plant; 4, an asymptomatic plant; 5, water control. Arrow points to RT-PCR product for WVMV.

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## DIFFERENTIAL DETECTION OF PRUNE DWARF VIRUS AND PRUNUS NECROTIC RINGSPOT VIRUS IN THE NURSERY ANNUAL VIRUS SURVEY

YunPing Zhang, Deborah Mayhew, and Umesh Kodira

Prune dwarf virus was first discovered in plum and prune. The virus causes many diseases among stone fruit crops such as dwarf of Italian prune, yellows of sour cherry, blind wood and narrow leaf of sweet cherry. The virus particles are isometric to short bacilliform up to 73 nm long.

Prunus necrotic ringspot virus was first discovered on peach in 1941 and many strains of the virus has been described since. These include almond calico, apricot line pattern, cherry necrotic ringspot, cherry rugose mosaic, Prunus ringspot, plum line pattern, stone fruit ringspot, sour cherry necrotic ringspot, and tater leaf of peach and cherry. Along with PDV, it also causes stunt in peach. The virus particles range from isometric to bacilliform up to 70 nm long. Both viruses are very wide spread due mainly to their ease of transmission through budding or grafting, seeds, and pollen.

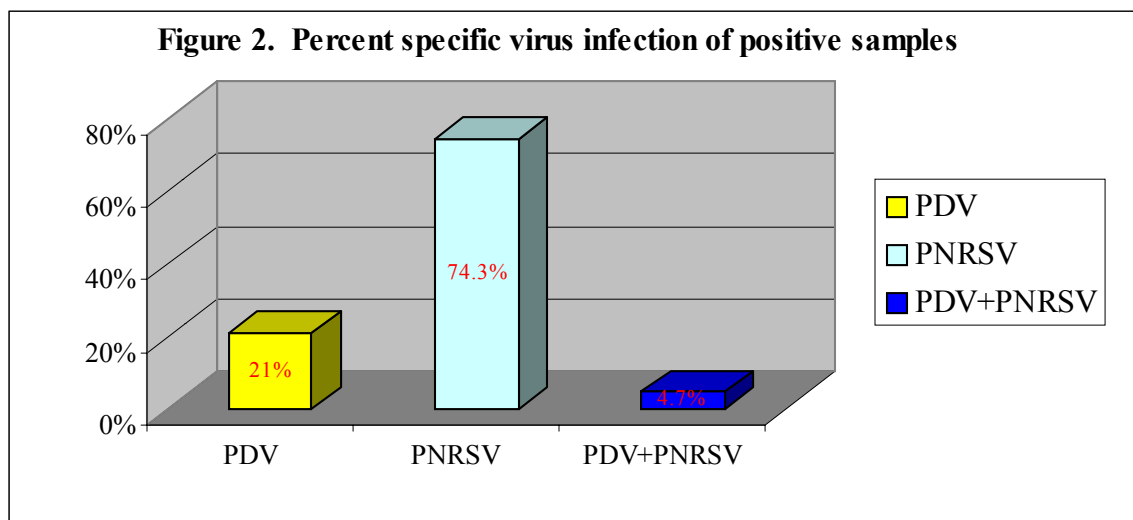
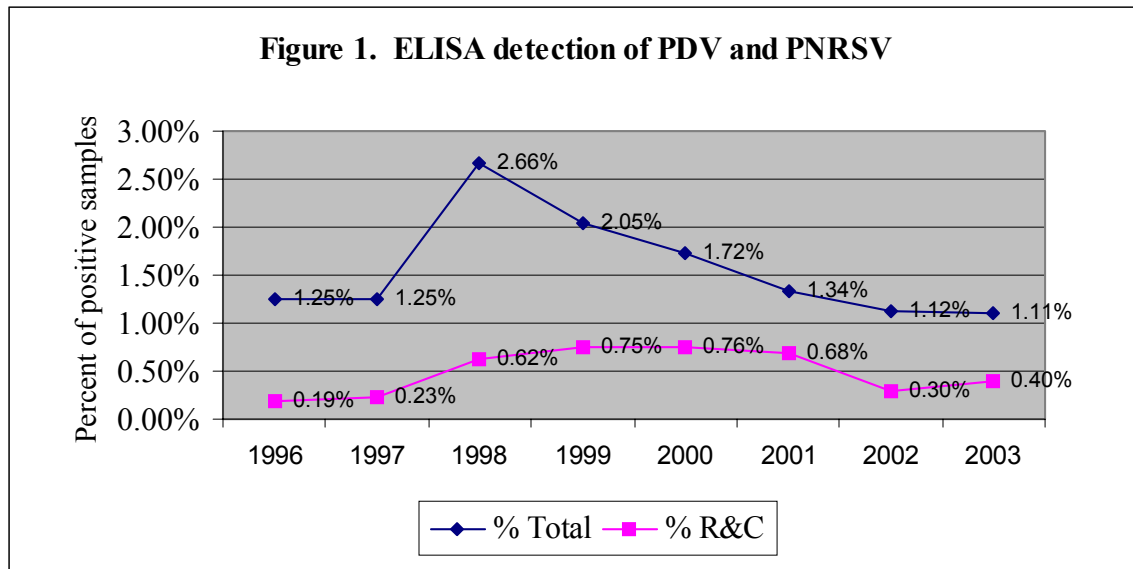
The only effective way to prevent these viruses from spreading in stone fruit crops is through the use of healthy material. Therefore, California Department of Food and Agriculture established the Nursery Program to test propagating source material annually for these viruses. In order to conserve resources and test large number of samples, these two viruses are tested in a combined single test without the determination of specific virus identity. We intended to investigate which virus is more prevalent in the nursery material.

The regular combo test is performed using polyclonal antibodies of both viruses as trapping antibodies and monoclonal antibodies as probe in a double antibody sandwich ELISA test. Samples positive for either virus or both are then re-tested in ELISA with PDV and PNRSV separately to determine which virus is present in the sample.

A total of 47,684 stone fruit tree samples were tested for these two ilarviruses (40,058 R&C samples and 7,626 service samples) from 18 nurseries, which is a 3.7% increase from previous year. There were 529 (1.11%) samples tested positive for PDV and /or PNRSV (Figure 1). After retest for individual virus of each positive sample, 111 (21%) of which were infected with PDV, 393 (74.3%) with PNRSV, and 25 (7%) with both viruses (Figure 2). The result showed that PNRSV is more prevalent and with a small percentage of mixed infections of both viruses.

In the year 2003, we also tested 1194 grapevine samples from 16 nurseries for grapevine fanleaf nepovirus and 1500 grapevine samples from 11 nurseries for grapevine leafroll associated viruses.

Acknowledgements: This project is supported by California Fruit Tree, Nut Tree, and Grapevine Improvement Advisory Board, Pest exclusion biologists, and participating nurseries.



## PLANT BACTERIOLOGY REPORT FOR THE YEAR 2003

Dan Opgenorth

The work of regulatory significance in the Bacteriology area in 2003 was interesting and rather exciting.

Real Time PCR detection of Citrus Canker (see figure 1) expanded to include other hosts in the Rutaceae Family (Sechwan Pepper and several California natives) and culminated in a poster presentation at the national meeting of the American Phytopathological Society (APS).



Opgenorth Figure 1: Citrus Canker lesions on dry leaves (photo by D. Opgenorth)

An overview of the International collection of Plant Pathogenic Bacteria (ICPPB) and the University of California Berkeley Collection of Plant Pathogens the (UCBPP) was initiated.

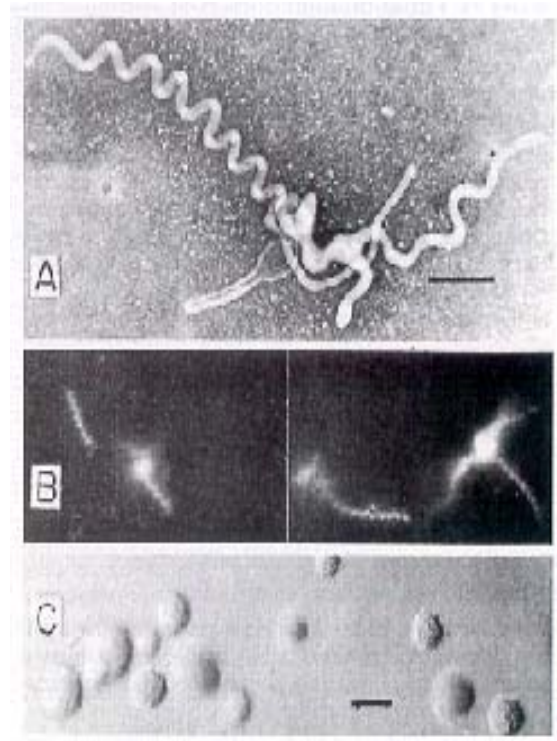
*Ralstonia solanacearum* race 3 biovar 2 was found on Geranium cuttings in several propagation greenhouses in the eastern part of the nation. This prompted a California survey and participation of our laboratory in diagnostic efforts. The initial alarm concerning the detection of *Ralstonia solanacearum* r3 b2 prompted some immediate

response from our laboratory. Since this was a new diagnostic event, cultures were needed for comparison. There were no isolates found in the CDFA collection, however. The ICPBP had 67 entries and the UCBBP had 12. Of these cultures, 31 and 7 were viable and 2 and 4 were respectively found to be *Ralstonia solanacearum* using BIOLOG. The Agdia dip stick Bid test was positive on 5 and 6 cultures from both respective collections. All isolates sent to David Norman at University of Florida for conformation were determined to be of biovar one, which was not the regulated pathogen of concern. This work allowed an in-depth evaluation of our *Ralstonia* isolates in the various collections and will provide a certain level of positive controls with which we can use to evaluate the selective media, Agdia Bid test and use of the BIOLOG identification system. Thus, the procurement and maintenance of the Berkeley collections has been shown to be of considerable diagnostic value. This will continue to be true as it becomes increasingly more difficult to get the necessary permits to acquire new cultures. However, as DNA technology improves, the collections could provide the resource for an electronic data base (BIOLOG-Bacterial Barcodes) that could easily be stored and utilized by other pathologists on the network. This is already a reality for some human bacterial pathogens.

Corn Stunt disease (figure 2) incited by *Spiroplasma kunkelli* (Figure 3) is still going strong in the central valley. This disease reduces the tonnage of silage corn by up to 50% in some cases. Since the corn leafhopper which transmits this disease has been found in Sacramento and Yolo Counties last year, we now anticipate the arrival of the problem for local growers. This year our laboratory worked with University of California Cooperative Extension (UCCE) Farm Advisors in Kings, Tulare, and Fresno Counties to confirm the diagnosis of the disease using antibody ELISA methods and also DNA based PCR techniques. Last season Dr. Charles Summers of UCCE at Parlier was able to determine that corn leafhoppers could over-winter as adults and nymphs in the base of the whorl of volunteer corn. However, it is still not clear if the *Spiroplasma* that incites the disease over-winters in the leafhoppers or the corn plants. While it is easy to get enough tissue for testing from the phloem of the corn, the small size of the hoppers presents a problem. In the past we needed to use five to ten adult insects to extract enough DNA for a PCR determination. However, laboratory technician, Raj Randhawa, has been able to scale down the extraction so that single leafhoppers can be used. This should help in our evaluation of the true over-wintering sites of the pathogen. We have also done much work in the evaluation of the detection of the disease in the respective parts of the corn plant. During the growing season, midribs of the symptomatic leaves are good initial sources, followed by the tassel, and finally the roots. It is interesting that the fibrous roots seemed to have the most *spiroplasma* and to be the most reliable source. Thus, we used this to evaluate some of Dr. Summers' field trials designed to test the use of insecticides to prevent or curtail the spread of the disease. While results are inconclusive, we did realize that there are some border effects in the plot. Therefore, the use of exterior trap plants with systemic pesticides may help to protect the interior of an entire field. At least the grower may have some time to get the crop better established before the onslaught of disease-transmitting leafhoppers. The use of cultural measures for control of this problem is certainly not absolute, but given that the disease seems to be endemic in the central valley, some degree of mitigation needs to be considered.



Opgenorth Figure 2: Corn with Stunt. The disease often resembles drought. Also note red leaves & multiple ears. Photo by D. Opgenorth.



Opgenorth Figure 3: Corn Stunt Spiroplasma A: electron micrograph B: fluorescent microscopy C: growth on agar plates. Photo by Robert Davis, USDA.

In addition, we were again called upon to participate in the Karnal Bunt survey and wheat testing project in the Palo Verde Valley of Riverside County. And finally, a total of 18 State permits were evaluated which concerned the movement, importation, or work with plant pathogenic bacteria in California.

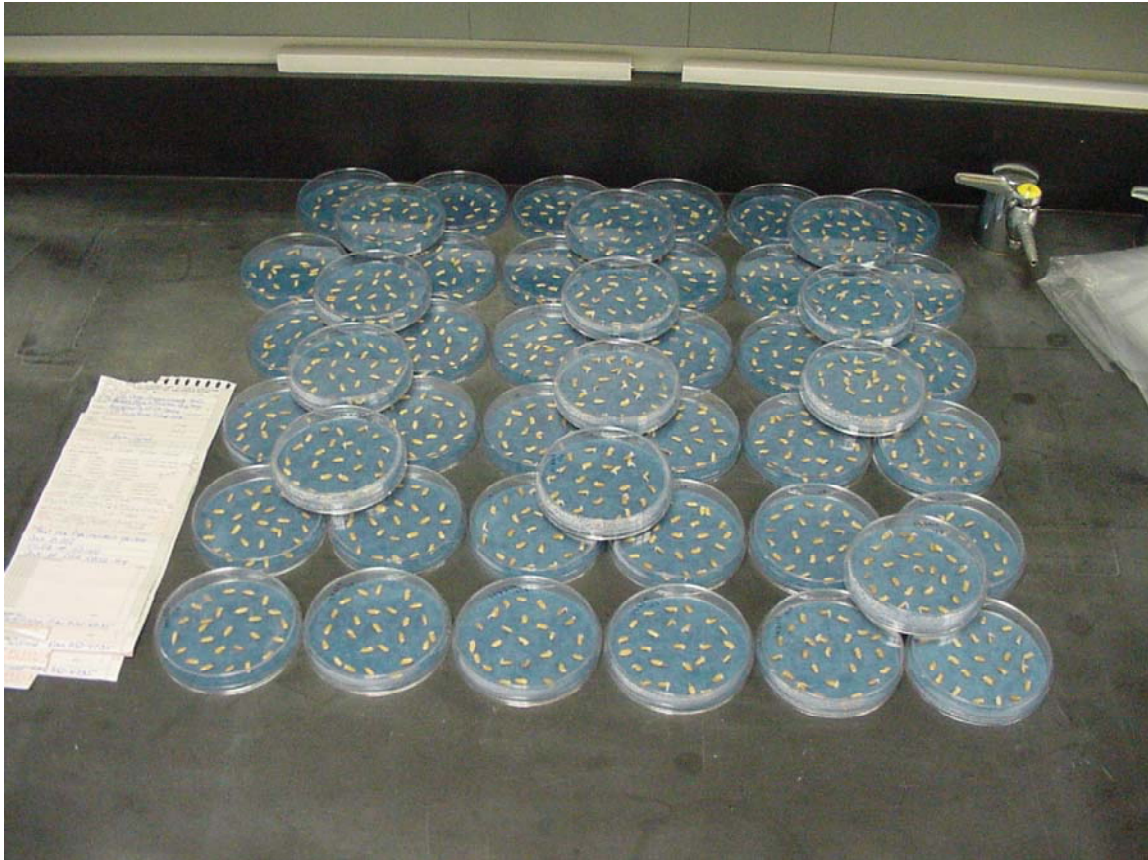


## SEED HEALTH TESTING

Timothy Tidwell, Allen Noguchi, Diana Fogle,  
YunPing Zhang, Jeanenne White, and Alex Ballesteros.

### Seed Health Testing Program

The Seed Health Testing lab ran approximately 470 seed health tests in 2003, involving 19 different types of agricultural or horticultural seed (figure 1). Thirty-four different clients in the seed industry were served, and cost recovery fees totaling more than \$33,000 were collected.



Tidwell et al. Figure 1. An example of a Seed Health Test for detection of the Rice Blast Pathogen, *Pyricularia grisea*. Photo by T. Tidwell.

The seed health laboratory staff also participated in county seed inspector training at various training sites in California in the spring of 2003, and a presentation on Seed Health Testing Techniques as well as the National Seed Health System was given at a national meeting of the Association of American Seed Control Officials (AASCO) in summer of 2003.

Plans were made for Seed Health Testing staff to participate in the Annual CDFA Seed Laboratory training of Registered Seed Technologists in 2004. The topic, by request of seed technologists, will be troubleshooting seed mold problems in laboratory germination tests.

Contributions from Seed Health testing staff were made to the National Seed Health System (NSHS). Seed health testing protocols were reviewed which are under consideration for inclusion in the USDA's Reference Manual B, which contains the approved protocols for seed health testing that are used in the United States for seed being exported. In addition, Tim Tidwell, who is a USDA-certified NSHS auditor, performed an accreditation audit of a California based seed health testing laboratory in Woodland, CA. Consequently this private seed health laboratory is now accredited by the USDA and the NSHS to conduct several different seed health tests, the results of which can be used as the basis for the USDA to write Phytosanitary Export Certificates. More NSHS audits of seed health testing laboratories are anticipated in 2004.

The staff of the Plant Pest Diagnostics Seed Health Testing Laboratory partnered with staff from CDFA's Center for Analytical Chemistry in 2003 to research a solution to a problem in evaluating the "moldiness" of cotton and almond seed coming through CDFA's Inspection Services Branch. The Chemistry lab was assigned the task of figuring out a way to quantify the degree of "moldiness" of seed samples. Through research, the PPDC Seed Health staff developed a procedure using a dilution plating technique to accomplish this task. The resulting procedure was able to be used as a means of quantifying the degree of "moldiness" of seed samples, based on the number mold propagules detected per weight of sample. Furthermore, this protocol provided the CDFA Analytical Chemistry Lab staff with a useable standard by which to compare and evaluate commercially available and user-friendly "mold detection kits" for their accuracy and feasibility in this unique application.

Wheat was again tested for the Karnal Bunt Pathogen, *Tilletia indica*, at the USDA laboratory in Blythe, CA. A few positive fields were detected during the 2003 season, based on the presence of diseased kernels in wheat samples taken from those fields. Thus, unfortunately for wheat growers, the Karnal Bunt pathogen was confirmed to still be alive, well, and active in the California's Palo Verde Valley, albeit in only localized areas, rather than widely scattered throughout the Valley. Additional survey and testing will take place in the 2004 season in this same area of California.



## MYCOLOGICAL PLANT PEST DIAGNOSTICS

Timothy Tidwell and Diana Fogle

In addition to the routine sample diagnostics, a number of new diseases to California were studied. *Phoma exigua*, a new pathogen of lettuce, was the subject of a mycological study by Diana Fogle, in conjunction with researchers from the University of California Cooperative Extension (UCCE). The disease (Figure 2) has become established in the coastal lettuce growing region and has caused serious economic losses, particularly to romaine lettuce crops. The research resulted in a poster presented at the 2003 national meetings of the American Phytopathological Society (APS). The poster was entitled “Phoma Basal Rot of Lettuce caused by *Phoma exigua*” by S.T. Koike, K.V. Subbarao, G.L.M. Verkley, T.M. O’Neil, and Diana Fogle.



Tidwell et al. Figure 2: *Phoma exigua* basal rot of lettuce. Photo by Steven Koike, UCCE.

A new lily disease (Figure 3) to California lily growers was also studied in 2003. Initially discovered to be a serious disease of Asiatic lilies in Santa Barbara and San Luis Obispo Counties by County staff, it was determined that this aggressive fungal pathogen was indeed new to the North American continent, having only been previously known in parts of Europe. This research resulted in a journal publication, H.J. Scheck, D.G. Fogle, and T.E. Tidwell. Plant Disease 87:1396, 2003. First Report of *Botryotinia* Blight of Asiatic Hybrid Lily Caused by *Botryotinia sphaerosperma* in North America.



Tidwell et al. Figure 3. *Botryotinia* Blight of Asiatic Hybrid Lily Caused by *Botryotinia sphaerosperma* Photo by Scott Kinnee, CDFA.

A new disease of Foxglove (*Digitalis* spp.) to California, caused by *Ascochyta verbasci* was also a subject for study at the PPDC mycology laboratory. This pathogen causes a leaf spot disease that was initially discovered by a Santa Cruz County biologist on foxglove plants grown from seed on-site at a local community college for use in campus landscaping. Relatively little is known about the host range and biology of this pathogen. From grow-out seed health tests of the unused seed it was determined that the pathogen was apparently not introduced via the seed, which was from Germany. The source of the pathogen in Santa Cruz has yet to be determined. Research is ongoing with this disease.

One other new disease that is currently under study is an anthracnose disease of the ornamental, *Jartropha* sp., caused by the fungal pathogen *Colletotrichum capsici*. This pathogen is commonly found in the Southeast USA on vegetables such as peppers, but is not reported in California. Initially detected in a San Luis Obispo County nursery by County biologist Kirk Schramm, research on this disease is also ongoing.

PPDC mycology staff received training in the recognition of soybean rust pathogens, *Phakopsora meibomiae* and *Phakopsora pachyrhizi*, at the USDA APHIS facility in Fort Detrick Maryland. This training was provided by the National Plant Diagnostic Network (NPDN) which is a branch of the new federal Homeland Security Department. The

NPDN is involved in the diagnosis of high-profile agricultural pests, sometimes referred to as potential agents of “bio-terrorism.” Various other NPDN proposals were reviewed which involved rice diseases and tree fruit diseases.

The PPDC Mycology staff also served on a scientific review committee in the long overdue task of assigning several Q-rated pathogens permanent ratings. This resulted in the successful assignment of permanent ratings for a number of pathogens such as the Daylily rust pathogen, *Puccinia hemerocallidis*; the groundsel rust pathogen, *Puccinia lagenophorae*; the rice blast pathogen, *Pyricularia grisea*; the Dutch Elm Disease Pathogen, *Ophiostoma ulmi*; Wisteria Vein Mosaic Virus (WVMV); and the bougainvillea leaf spot pathogen, *Cercosporidium bougainvilleae*.

## EPIDEMIOLOGICAL ASSESSMENTS OF PIERCE'S DISEASE

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**Abstract:** Vineyards in the 7 grape production areas of Kern county's area wide management project for 2002-2003 were surveyed for PD. Incidence of PD in the highly affected areas (General Beale and North) peaked in 2002, and declined dramatically in 2003. Treatments to reduce GWSS and to identify and remove PD infected vines each year was associated with these reductions. A representative General Beale vineyard is mapped for years 2001 – 2003. An epidemiology data processing center was established at CAMFER at U.C. Berkeley.

### INTRODUCTION:

This project was conducted cooperatively with a Kern County project with Jennifer Hashim. These two projects have complimentary objectives and methods, and by combining people and resources we achieved a synergistic efficiency.

The epidemiology of Pierce's Disease (PD) changed dramatically in California with the arrival of the glassy-winged sharpshooter (GWSS) about 15 years ago. Before that time the disease caused losses, but the damage accumulated gradually resulting in the loss of a small percentage of vines. With the arrival of the GWSS, however, PD spread has increased logarithmically, such that entire vineyards were destroyed in as little as 3 to 5 years. In Kern Co. where the disease was previously inconsequential, PD may now threaten more than 88,000 acres of grape production. To cope with this development there have been extensive field studies to determine effective methods to control the insect vector, the GWSS. However our understanding of how to control the disease and the characterization of the changes in the epidemiology of PD when the causal bacterium is transmitted by GWSS has been based on limited field data.

The cooperative area-wide pest management of the GWSS project has defined 7 distinct grape growing areas in Kern County. These areas are currently at various "stages" in the PD epidemic, ranging from the General Beale area--where GWSS was first observed in 1997 and where the epidemic occurred first and has been most severe--to the Highway 65-Delano area where GWSS was first observed in 2001 and where there is still very little PD. This variation among growing areas in combination with the significant accumulation of field data about these areas makes Kern County an ideal area to locate epidemiological projects. Extensive data has been obtained about GWSS populations and the effectiveness of various treatments in controlling GWSS. These two collaborative projects are obtaining data about the incidence of PD over time in each area, and the control measures and possible epidemiological factors that may affect the epidemic.

**OBJECTIVES:**

- A. Determine changes in the incidence of PD over time in seven distinct grape-growing areas in Kern County.
- B. Develop PD monitoring and management techniques and strategies for use by growers to reduce risk and damage. Update and provide educational materials to assist vineyard managers, pest control advisors, other researchers and government agencies involved in advising growers in the area-wide pest management of the GWSS project.
- C. Evaluate the importance of epidemiological factors such as GWSS population size, vine age, cultivar susceptibility, control practices, and GWSS control treatments in vineyards, and nearby GWSS hosts or habitat.
- D. Create a central data processing facility at the Center for the Assessment and Monitoring of Forest and Environmental Resources (CAMFER) on the UC Berkeley campus to compile the data from this project in a GIS format. Share the resulting data, maps, and information with collaborating plant pathologists, statistical analysts, agricultural economists, and other legitimate researchers.

**RESULTS AND CONCLUSIONS:**

Vineyards were monitored by visually inspecting each vine for PD symptoms and collecting and testing (by ELISA) samples from symptomatic vines. Tables 2 and 3 summarize the results for the 7 grape growing areas in Kern County. About 5% of Kern county's grape production acreage was monitored. The General Beale, north, central, south, and west areas have had GWSS since about 1997. In the General Beale and north areas the GWSS populations reached very high numbers in 2000-2001 (see Hill fig. 1), and the south, central, and west areas have had much lower but persistent populations. GWSS was detected in 2001 in the Hwy 65-Delano area. More than 10 vineyards in the General Beale area and more than 2 vineyards in the north area were severely impacted by PD in 2001 and 2002, with infection rates between 2% to more than 50%. Many of these were not included in this survey because sampling and testing the high number of infected vines would require more resources than was available. However fig. 2 presents the progression of the epidemic in a representative Red globe vineyard. The high infection rate in 2002 probably represents infections that were established in 2000 and 2001. The dramatic reduction apparent in 2003 is associated with a management program of area-wide GWSS reduction combined with roguing PD vines and replanting. This project developed effective methods and demonstrated that monitoring vineyards for PD, testing, removing infected vines, and replanting is very inexpensive when PD incidence is low, in the order of less than \$5 per acre per year.

All PD survey data from this project has been compiled in GIS and database formats by the Center for the Assessment and Monitoring of Forest and Environmental Resources (CAMFER) at University of California at Berkeley. This is the second in a projected five year project. In addition to the vineyards shown in tables 2 and 3, about 3000 additional acres in Kern and Tulare counties have been monitored. That data and the information from GWSS trapping surveys is being added to the data set at CAMFER. The resulting data, maps, and information will be made available to other scientists, government, and industry people involved in the management of PD in California.

A profile was created for each vineyard and the variables recorded include: GPS coordinates, cultivar, vine age/plant date, row and vine spacing, pruning and trellising system, weed index, proximity to other host crops of GWSS, pesticide use information when available, and presence and population levels of GWSS. Fifteen cultivars of varying ages were examined during the project to correlate respective tolerances to PD (Hill Table 1.). Analysis of temporal and spatial PD patterns and comparisons among the vineyards over time should lead to better models of PD epidemiology, a with quantitative estimates of how epidemiological variables, such as the incidence of PD combined with sampled populations of GWSS affect the further spread of PD. This understanding should lead to better control and management practices.

**Hill Table 1. Cultivars monitored in 2002-2003 for Pierce's disease. Vine susceptibility: 1=most tolerant, 2=less susceptible, 3=most susceptible, NA=unknown.**

<b>Green</b>		<b>Red</b>		<b>Purple/Black</b>	
Calmeria	3	Christmas Rose	NA	Autumn Royal	NA
French Colombard	2	Crimson Seedless	2	Black Emerald	NA
Jade Seedless	3	Flame Seedless	2	Fantasy Seedless	NA
Muscat	NA	Redglobe	3		
Perlette	NA	Ruby Seedless	2		
Thompson Seedless	1				
Superior Seedless	NA				

**Hill Table 2. Summary of the Monitoring for Pierce's disease in 2002**

Areas surveyed for PD	Number of vineyards	Number of acres/ Number of vines	Number of vines tested	Number of PD + vines Number PD+ vines per 1000
General Beale Pilot Area	41	849 ac 450991v.	2095	1238 PD+ * 2.75 +v./1000
North: Edison/Bena	7	159 ac 80769v.	159	116 1.44
South A: Arvin	21	304 ac 154208v.	46	9 0.058
South B: Arvin	28	261 ac 131247v.	74	7 0.053
Central: Arvin	5	55 ac 32631v.	5	0 0.0
West: Hwy 166	32	797 ac 375671v.	57	6 0.016
Hwy 65 and Delano	83	1636ac 790181v.	243	0 0
Total	216	4060ac 2015698v.	2543	1376 0.68

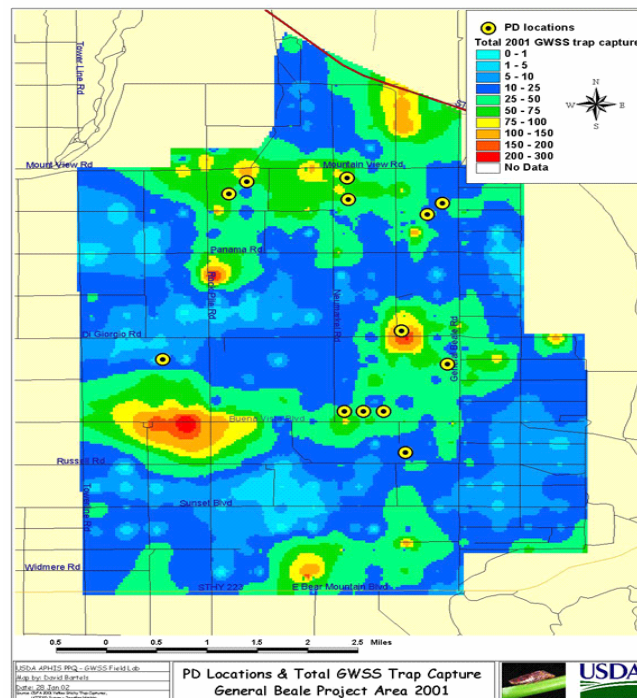
- 98.8% (1224 of 1238) of the PD positive vines in the General Beale area were in 2 out of 6 varieties, Redglobe and Crimson, on 113.4 acres and 40 acres respectively.

**Hill Table 3. Summary of the Pierce's disease survey effort in Kern County in 2003**

Areas surveyed for PD	Number of vineyards	Number of acres	Number of vines tested	Number of PD + vines Number PD+ vines per 1000
General Beale Pilot Area	41	849	326	188 PD+* 0.42 +v./1000
North: Edison/Bena	7	159	108	82 1.03
South A: Arvin	21	304	28	2 0.013
South B: Arvin	28	261	36	9 0.069
Central: Arvin	5	55	5	0 0.0
West: Hwy 166	32	797	99	22 0.065
Hwy 65 and Delano	83	1636	127	3 0.0038
Total	208	3958.63	729	306 0.152

\* 96.8% (182 of 188) of the PD positive vines in the General Beale area were in the same 153.4 acres of Redglobe and Crimson as in 2002.

**Hill Figure 1. PD locations\* and total GWSS trap captures in the General Beale Pilot Project area in 2001.**



\* PD locations are where PD vines were observed but not in all cases mapped nor the incidence quantified.

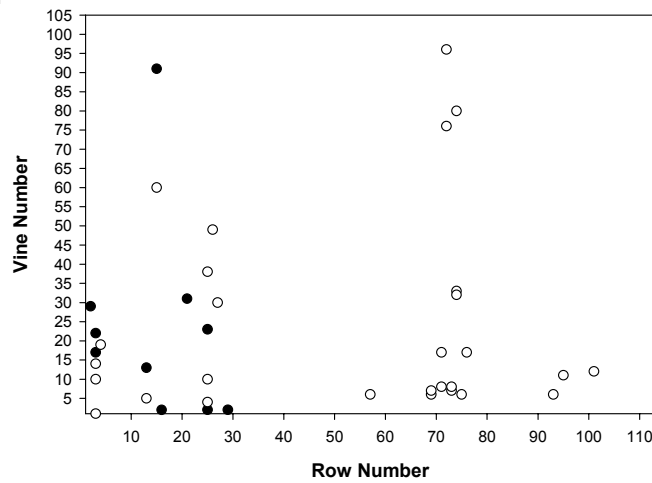
**Hill Figure 2. Three years results of vineyard survey in General Beale area.**

**2001 General Beale Vineyard**

Site ID: GB313016103  
**Total PD + Vines: 10**  
 All Positive vines rogued/replanted

Cultivar: Redglobe  
 Acres: 20  
 Plant Date: 1997  
 # Rows: 113  
 # Vines: 105  
 Training: Quad.-cordon  
 Trellis: Continuous gable

● 2001 + Sample  
 ○ 2001 - Sample

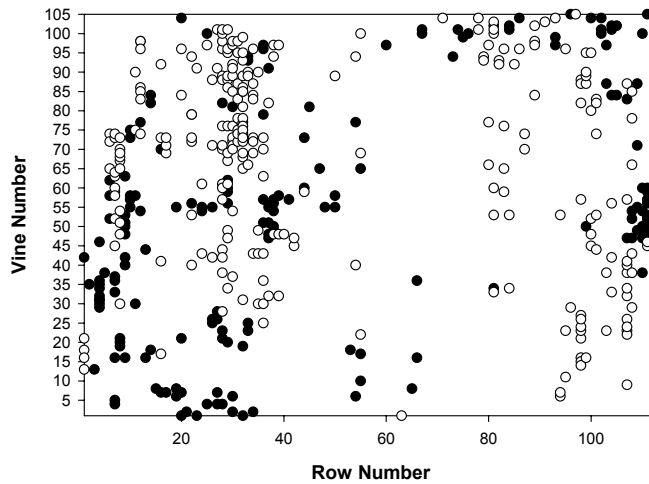


**2002 General Beale Vineyard**

Site ID: GB313016103  
**Total PD + Vines: 184**  
 All Positive vines rogued/replanted

Cultivar: Redglobe  
 Acres: 20  
 Plant Date: 1997  
 # Rows: 113  
 # Vines: 105

● 2002 + Sample  
 ○ 2002 - Sample

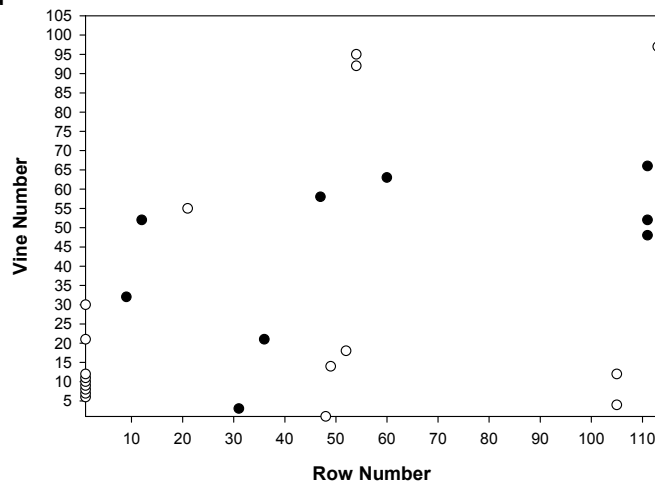


**2003 General Beale Vineyard**

Site ID: GB313016103  
**Total PD + Vines: 9**  
 All Positive vines rogued/replanted

Cultivar: Redglobe  
 Acres: 20  
 Plant Date: 1997  
 # Rows: 113  
 # Vines: 105

● 2003 + Sample  
 ○ 2003 - Sample



**FUNDING:** Funding for these two projects was provided by the University of California, Division of Agriculture and Natural Resources, Pierce's Disease Research Grants Program and the Pierce's Disease / Glassy-winged Sharpshooter Board.



# Sudden Oak Death

Cheryl Blomquist and Terra Irving

Sudden oak death caused by *Phytophthora ramorum* ( an Oomycete) is a disease found primarily in the wild lands of 12 California counties. This disease can kill tan oak and coast live oak, and in some locations, has caused widespread mortality in these oak species. This disease can also infect common nursery plants such as rhododendron and camellia. In response to the danger of moving this disease to new locations on nursery stock, federal and state quarantines regulate and require inspection and testing of nursery stock annually for nurseries that ship plants out of the infested area. There is also an ongoing effort by university researchers, forest service employees and county workers to survey the wild lands and map any change in the disease there.

Our laboratory at the PPDB receives all samples for *P. ramorum* testing in the 12 infested counties and samples from nurseries in all other California counties. We processed more than 1866 PDRs that were submitted for *Phytophthora ramorum* detection in 2003. Over 840 of the samples came from nurseries located in the infested and noninfested counties. Most of the nursery samples were taken by county biologists to comply with the yearly inspection requirement for nurseries located in infested counties that sell plant hosts of *P. ramorum*. This year, samples came from 2 source nurseries where *P. ramorum* was detected for the first time and one nursery which has had previous detections. *P. ramorum* was found on *Camellia sasanqua*, *Camellia japonica*, and *Viburnum tinus* for the first time in California (and the United States) as well as on rhododendron. Intense sampling followed these detections, with plants traced forward and traced back, inspected and tested. No new infected plants were found during the final 90 day inspection period for these nurseries. From the nurseries, 44 samples were identified with *P. ramorum*, 2 with *P. syringae*, 2 with *P. hibernalis*, and 1 with *P. citricola*. Last winter, *P. ramorum* was found on grand fir at a Christmas tree plantation and this year, *P. ramorum* DNA was detected on grand fir at a different plantation along the same ridge. At both these sites, *P. ramorum*-infected California bay laurel was overhanging the edge of the Christmas tree block and is probably the source of the disease in the grand fir.

Survey samples from the wild lands were submitted from a variety of groups. SODBusters (UC Forest Products Lab) submitted samples from the land surrounding their two areas which receive *P. ramorum*-infested wood products. Additionally, samples came from county biologists, USDA Forest Service workers, ground-checking groups from Cal Poly and Sonoma State, wildlife biologists, arborists and homeowners. From the wild lands, there were 288 *P. ramorum*, 54 *P. nemarosa*, 6 *P. pseudosyringae* and 2 *P. syringae* determinations.

The nursery diagnostic guide for *Phytophthora ramorum* being written by Steve Tjosvold (UC extension), Karl Buermyer (UC extension), Cheryl Blomquist (CDFA) and Susan Frankel (US Forest Service) is in progress, with a draft of the guide being used for the upcoming session to train nursery inspectors in late January. It will be published by the IPM group at University of California. Descriptions of *P. ramorum* on grand fir and a

new leaf spot disease in rhododendron caused by *P. hibernalis* are being submitted to the journal, *Plant Disease*.



*Camellia sasanqua* 'Bonanza' leaves infected with *Phytophthora ramorum*.



*Camellia japonica* 'Kamasaka' infected with *Phytophthora ramorum*. Notice the triangular-shaped lesions on the leaves and defoliation on the lower part of the plant.



*Phytophthora ramorum*-infected *Viburnum tinus* 'Compactum'. Notice the black stem lesion. In *Viburnum*, the infection can move from the leaves into the stems.

